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Fig. 1 Three-dimensional HPLC pattern of ASHMI

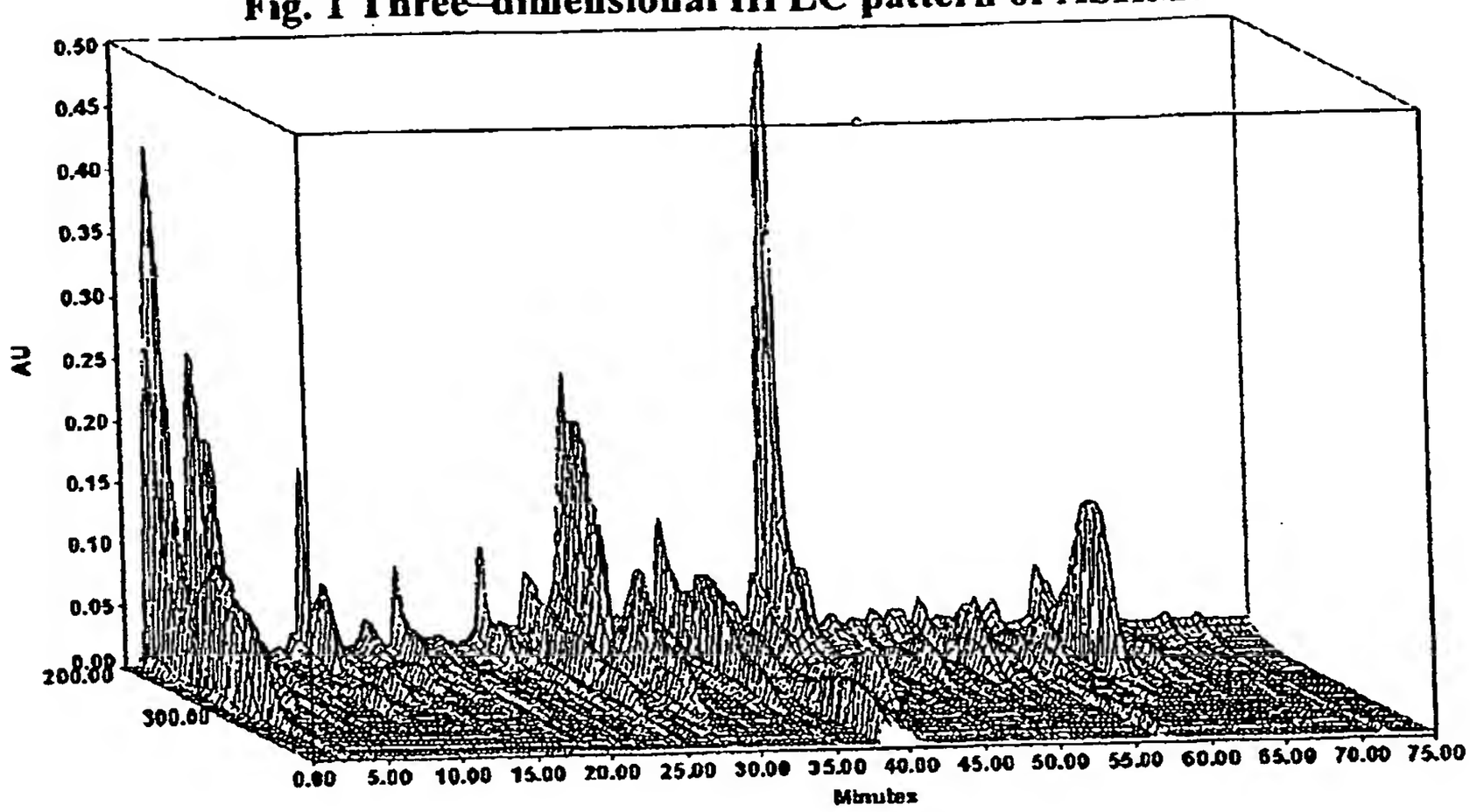


FIGURE 1

Fig. 2 The HPLC fingerprint of ASHMI at 254nm

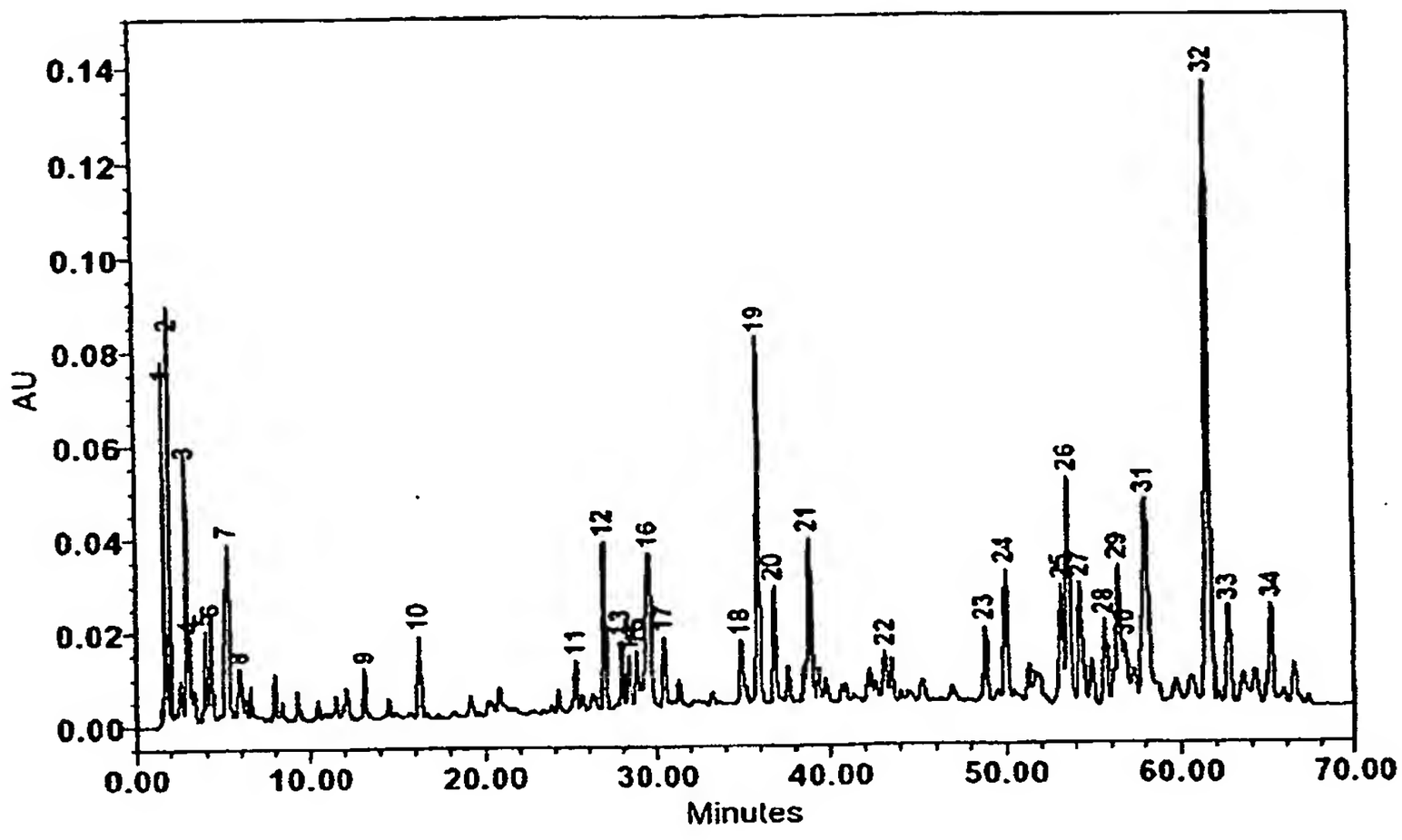


FIGURE 2

Fig. 3 Three–dimensional HPLC pattern of Ling-Zhi

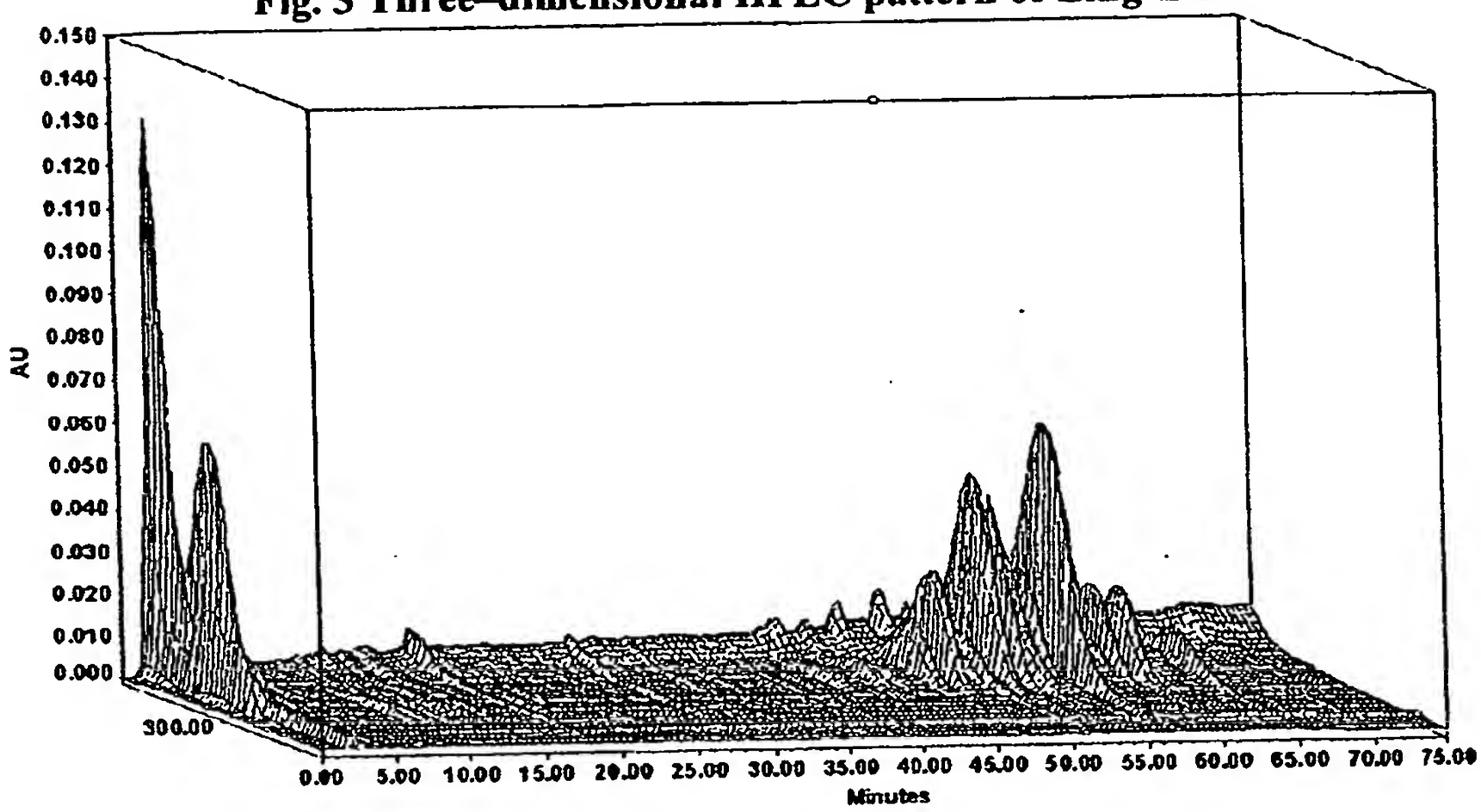


FIGURE 3

Fig 4 The HPLC fingerprint of Ling-Zhi at 254 nm

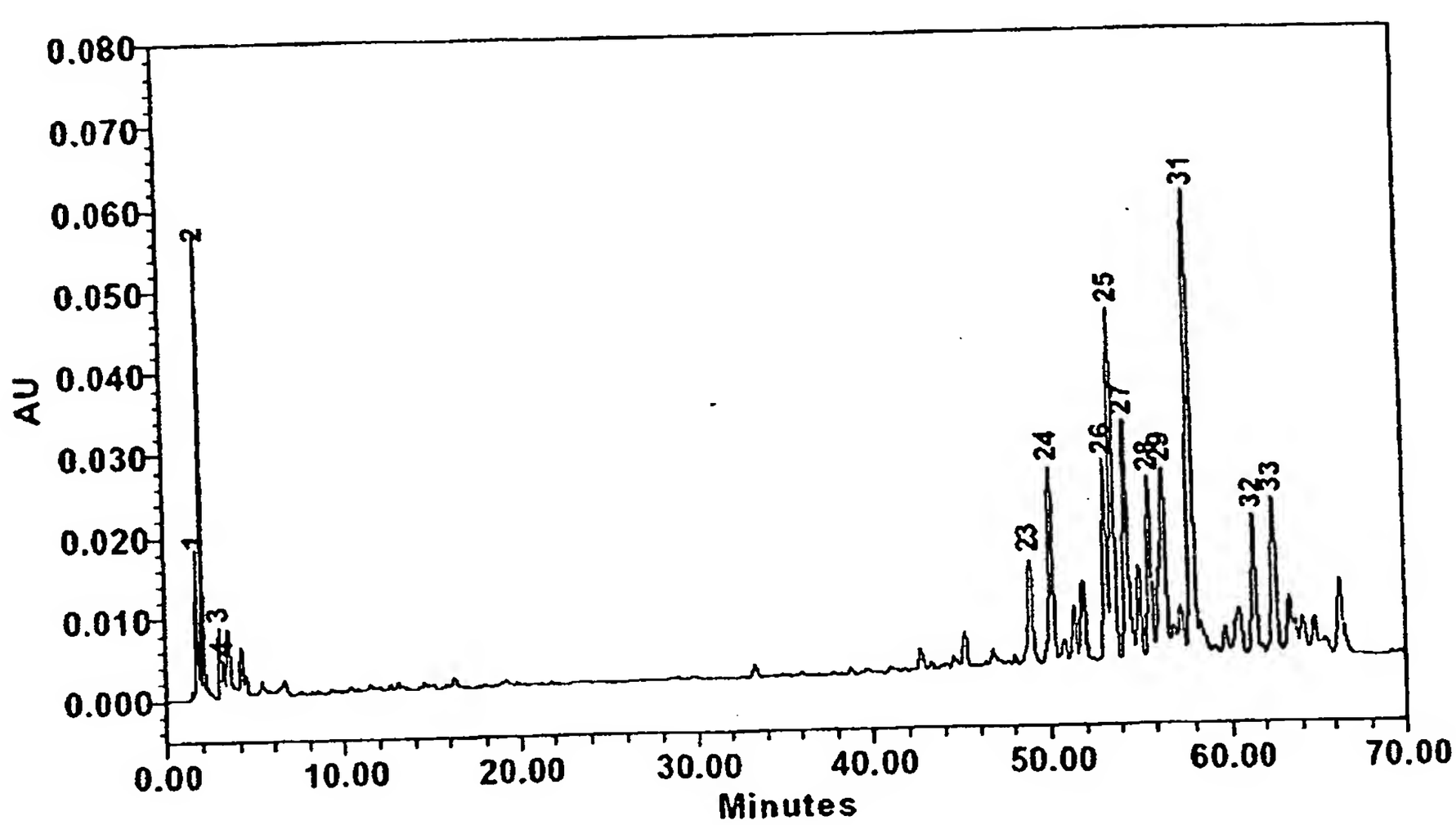


FIGURE 4

Fig. 5 Three-dimensional HPLC pattern of Ku-Shen

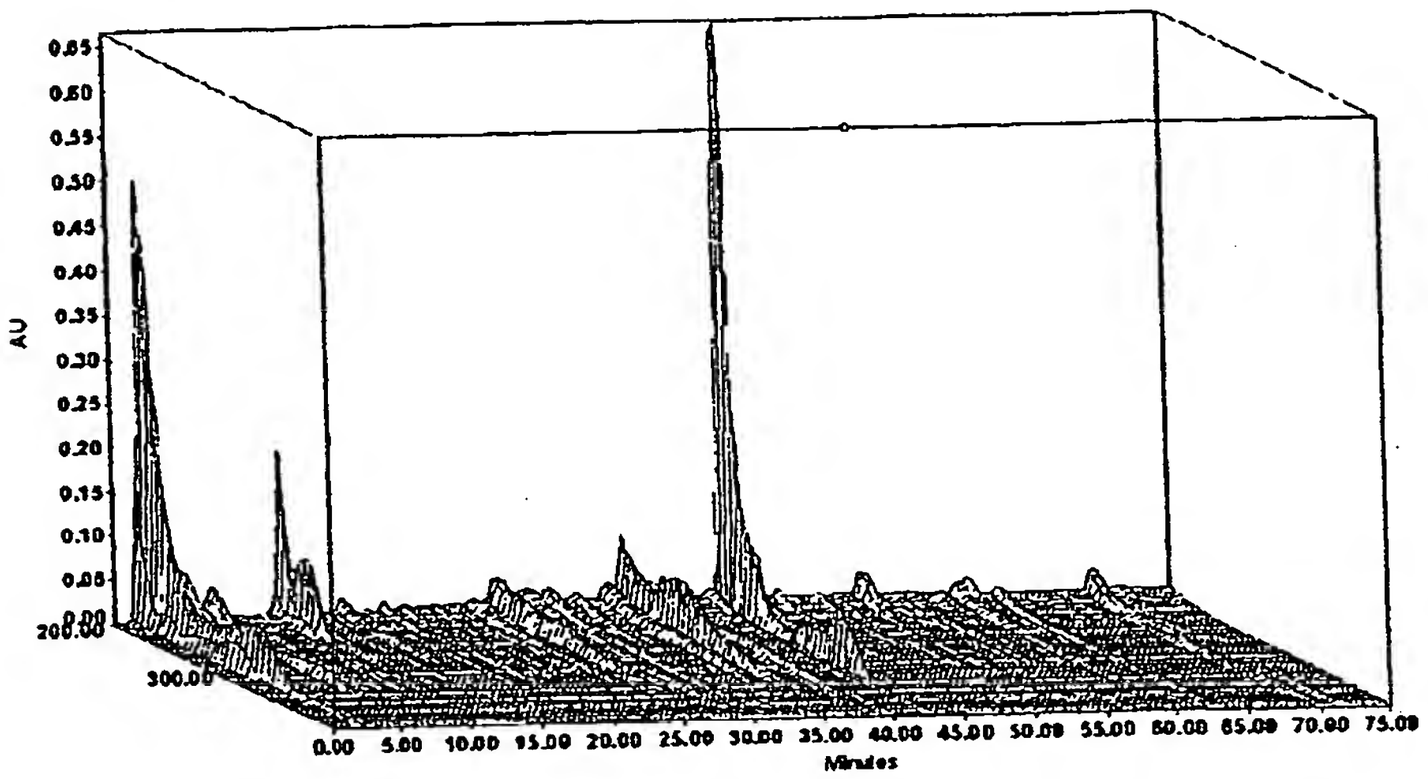


FIGURE 5

Fig. 6 The HPLC fingerprint of Ku-Shen at 254nm

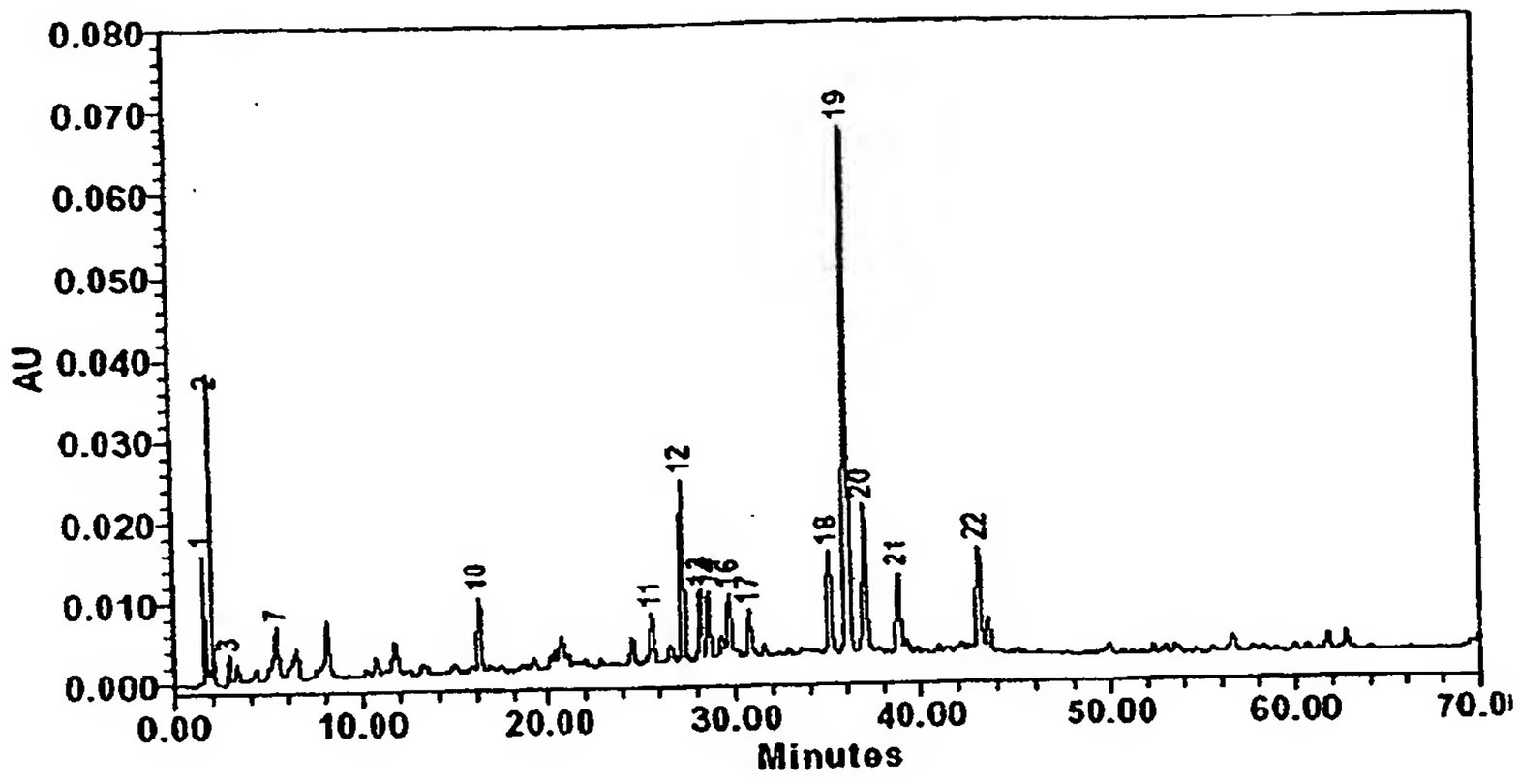


FIGURE 6

Fig.7 Three-dimensional HPLC pattern of Gan-Cao

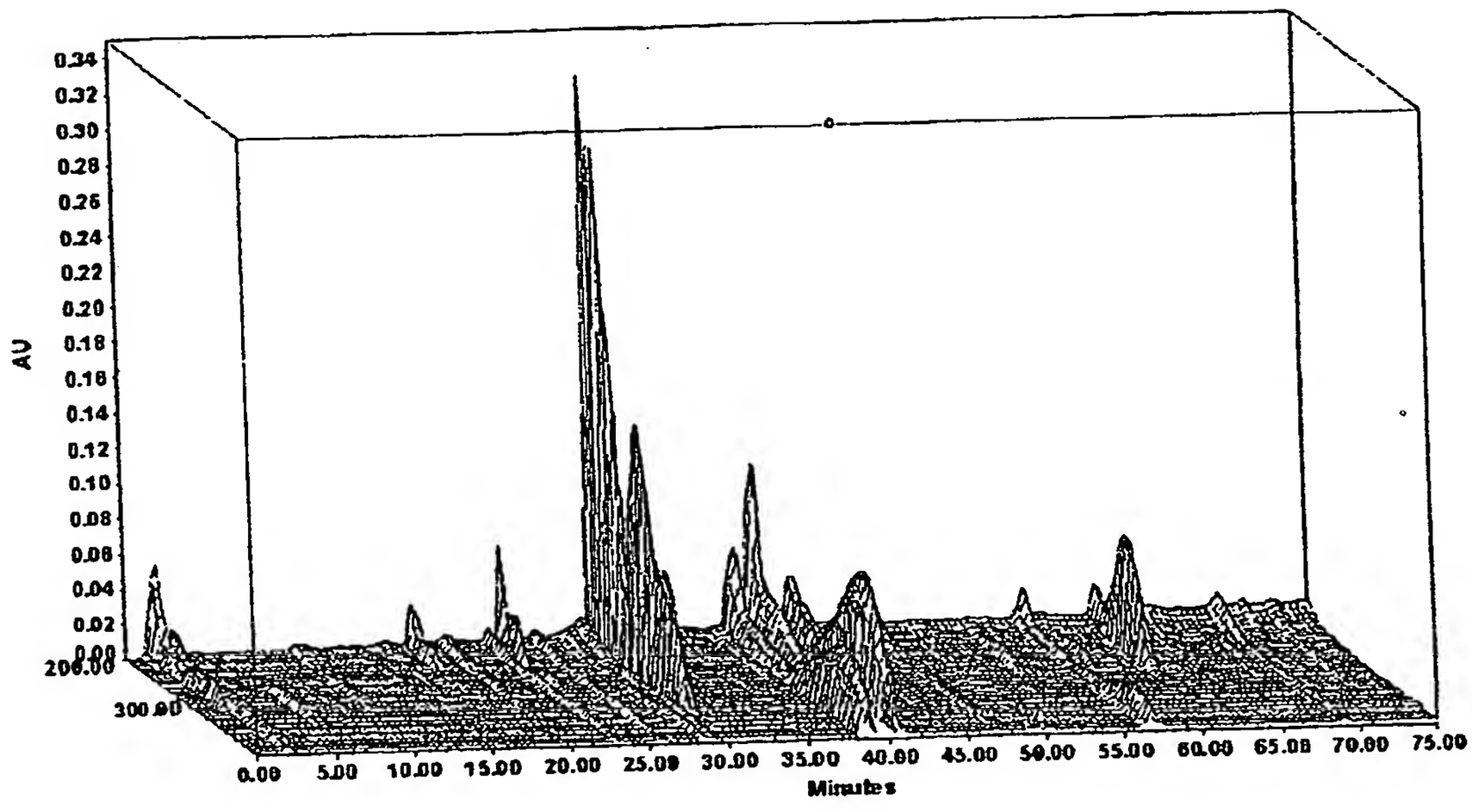


FIGURE 7

Fig. 8 The HPLC fingerprint of Gan-Cao at 254nm

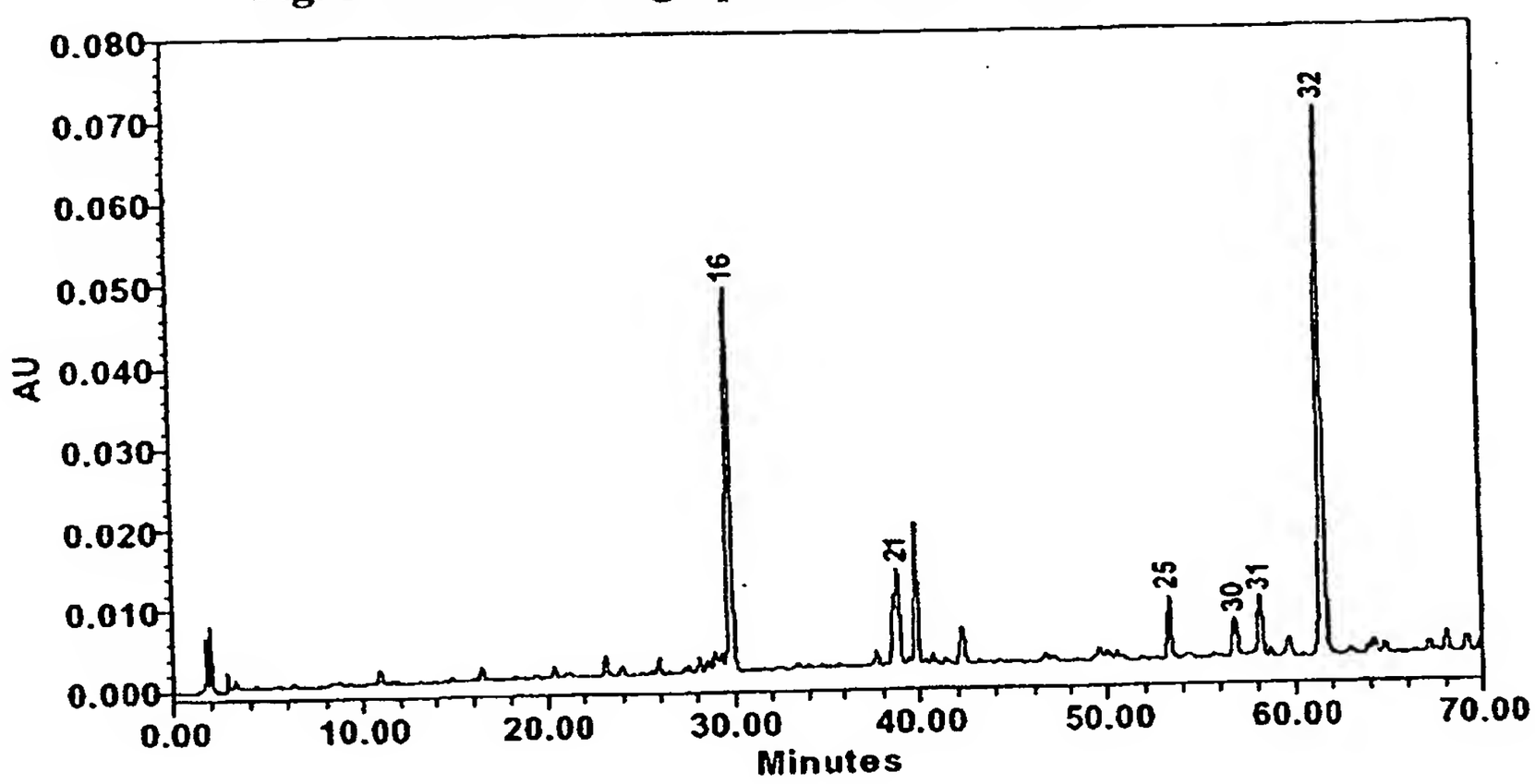


FIGURE 8

Fig. 9 The HPLC profiles of ASHMI and individuals (254nm)

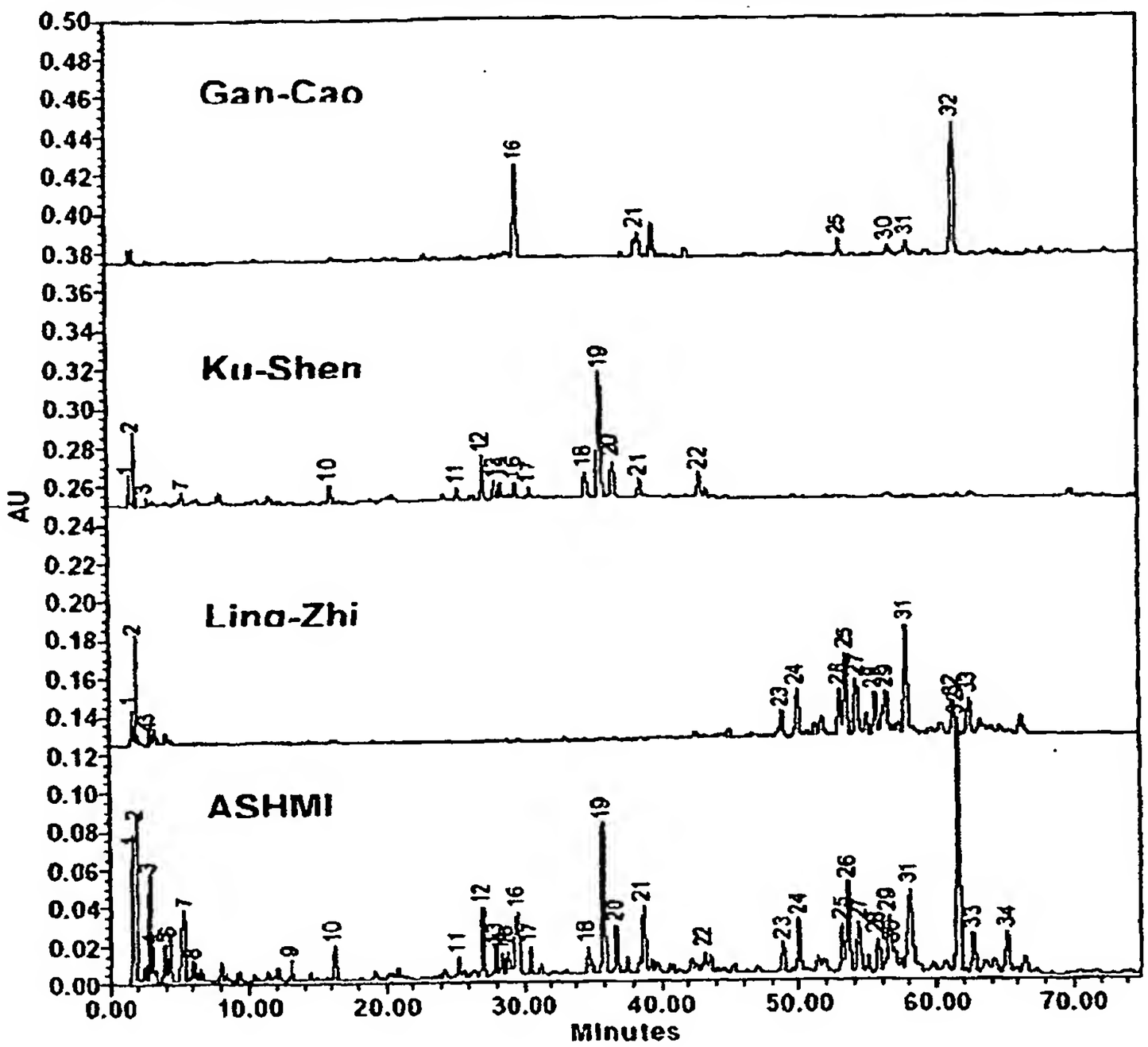


FIGURE 9

Fig 10. Protocol #1

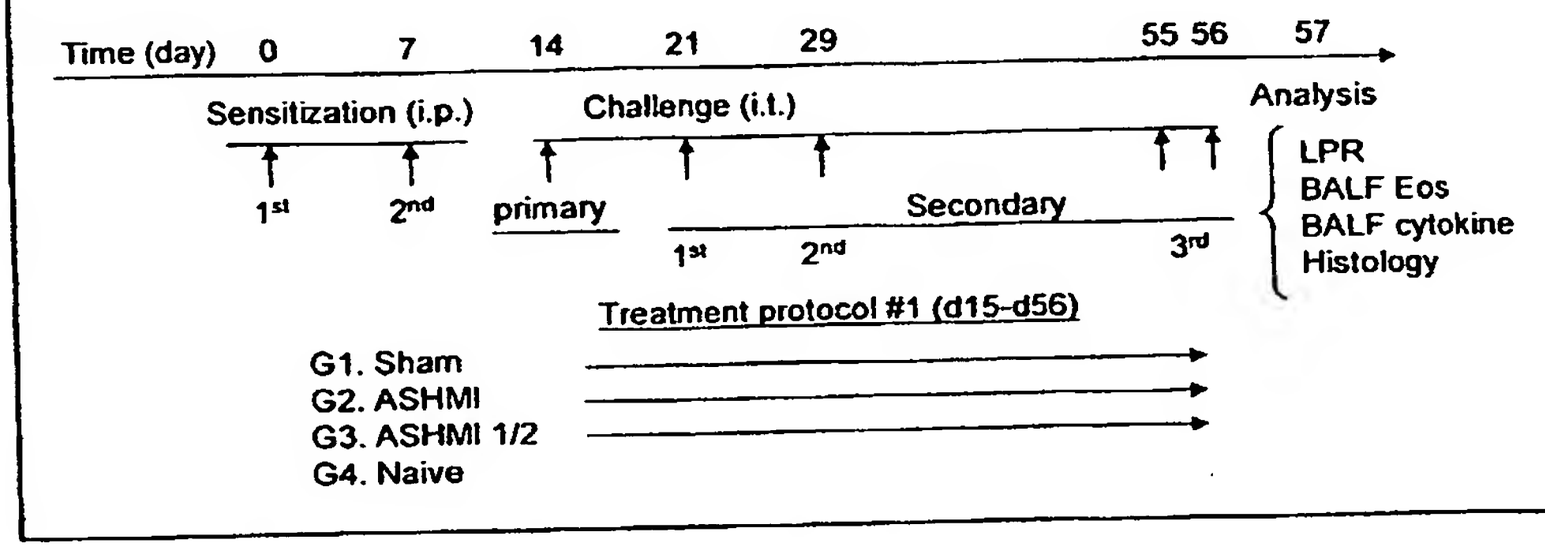


FIGURE 10

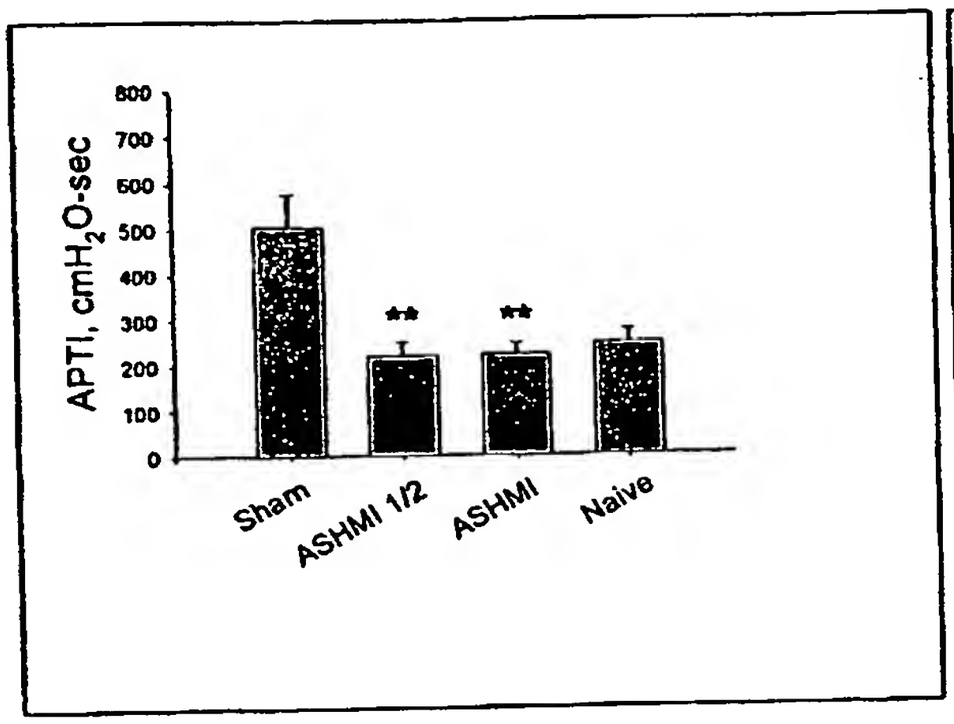
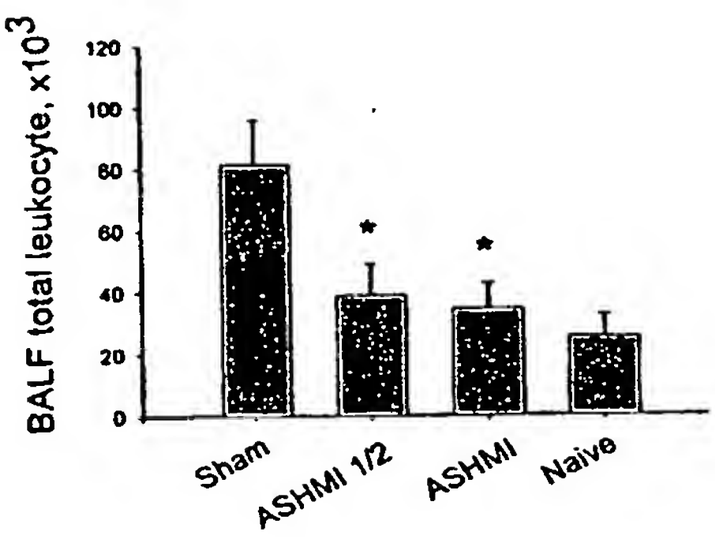


Fig 11. ASHMI treatment suppressed Ag-induced AHR. Data are Mean ± SEM of 6-8 mice from each group**p<0.01 vs sham.

FIGURE 11

A.



B.

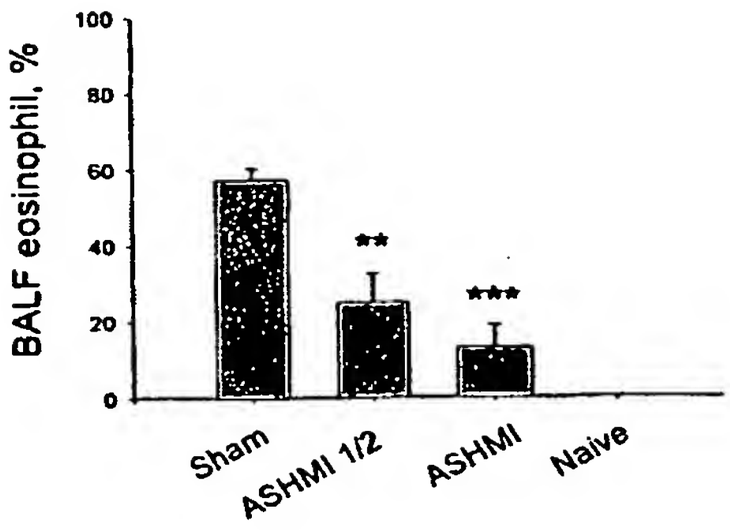


Fig 12. ASHMI reduced Ag-induced pulmonary inflammation: Total number of cells and differential counts of BALF cells were determined by microscopic evaluation. A. shows the total number of cells and B. shows percent of eosinophils. Data are Mean ± SEM of 6-8 mice from each group. *, $p<0.05$; **, $p<0.01$ vs sham and ***, $p<0.001$.

FIGURE 12

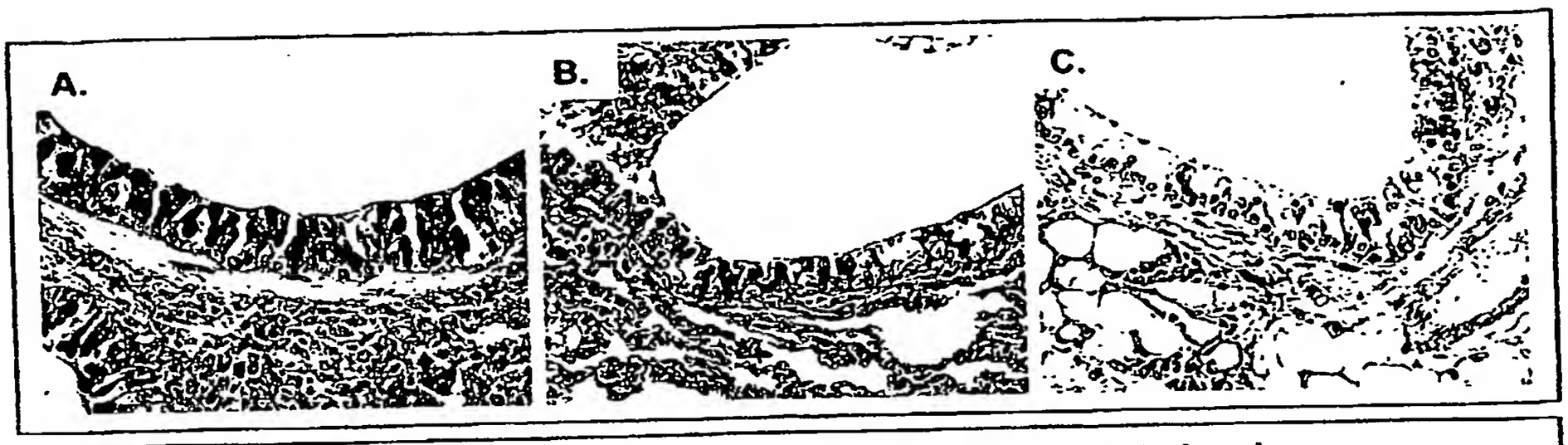


Fig4. Lung histology. Mice in each group (n=4/group) were necropsied after airway response measurement and unlavaged left upper lobe lungs were fixed in neutral buffered formaldehyde. Five-µm paraffin sections were stained with periodic acid-Schiff's reagent (PAS) for goblet cells. A. shows goblet cell hyperplasia in airway from a saline placebo treated mouse. B. illustrates markedly reduced mucus goblet cells in airways of ASHMI treated mice. C. shows absence of goblet cells in airways of naïve mice.

FIGURE 13

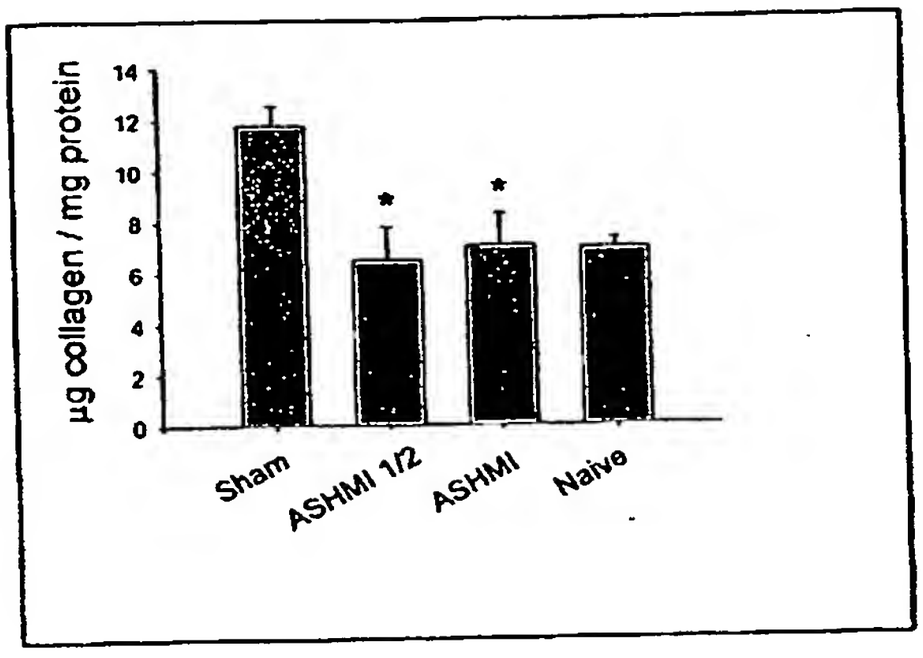


Fig 14. Decreased Collagen content. Data are Mean ± SEM of 4-5 mice from each group*, $p<0.05$ vs sham.

FIGURE 14

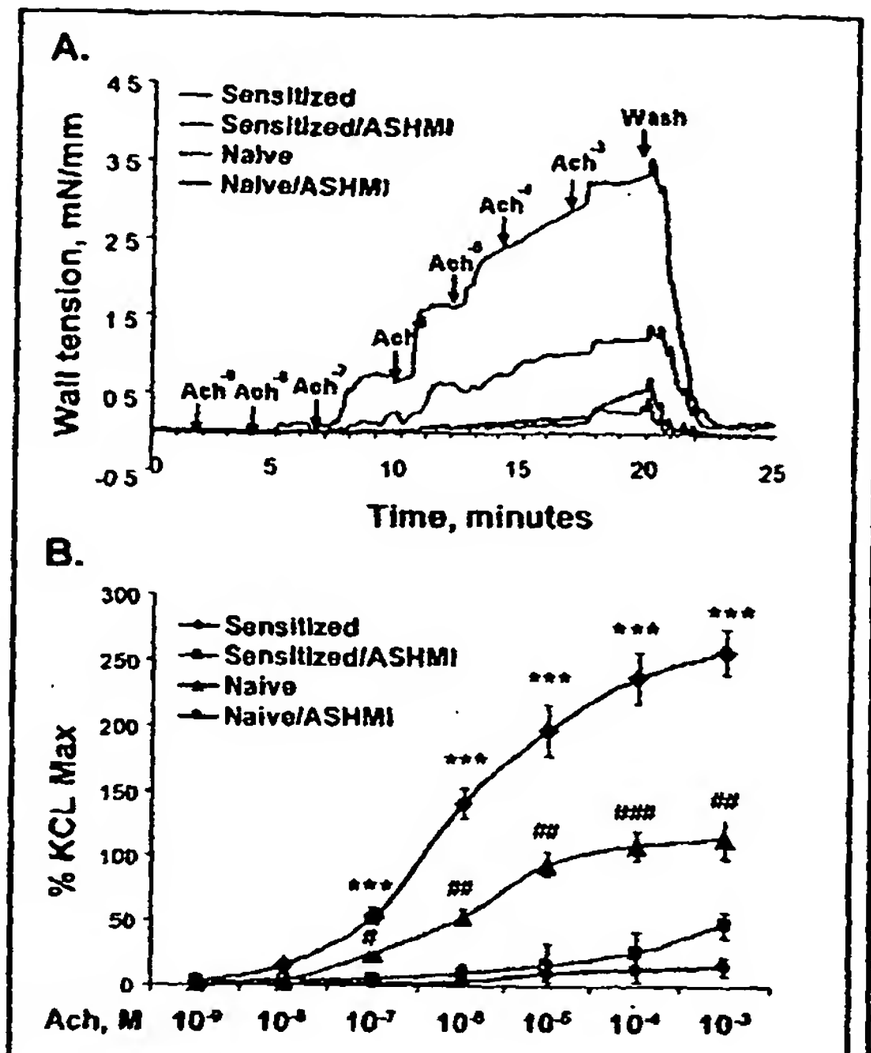


Fig 15. ASHMI inhibits contractile responses of tracheal ring to acetylcholine. Contractile responses to increasing doses of Ach were evaluated in tracheal rings in the presence and absence of 100 ug/ml ASHMI. (A) shows real-time tracing of a typical contractile response. Responses from 4 animals were used to generate a dose response curve to Ach (B). Data are expressed as percent of maximal contraction to 60 mM KCL.**, $p<0.01$; ***$p<0.001$ vs, Sensitized/ASHMI. ##; $p<0.01$; ###, $p<0.001$ vs Naïve/ASHMI.

FIGURE 15

FIGURE 16

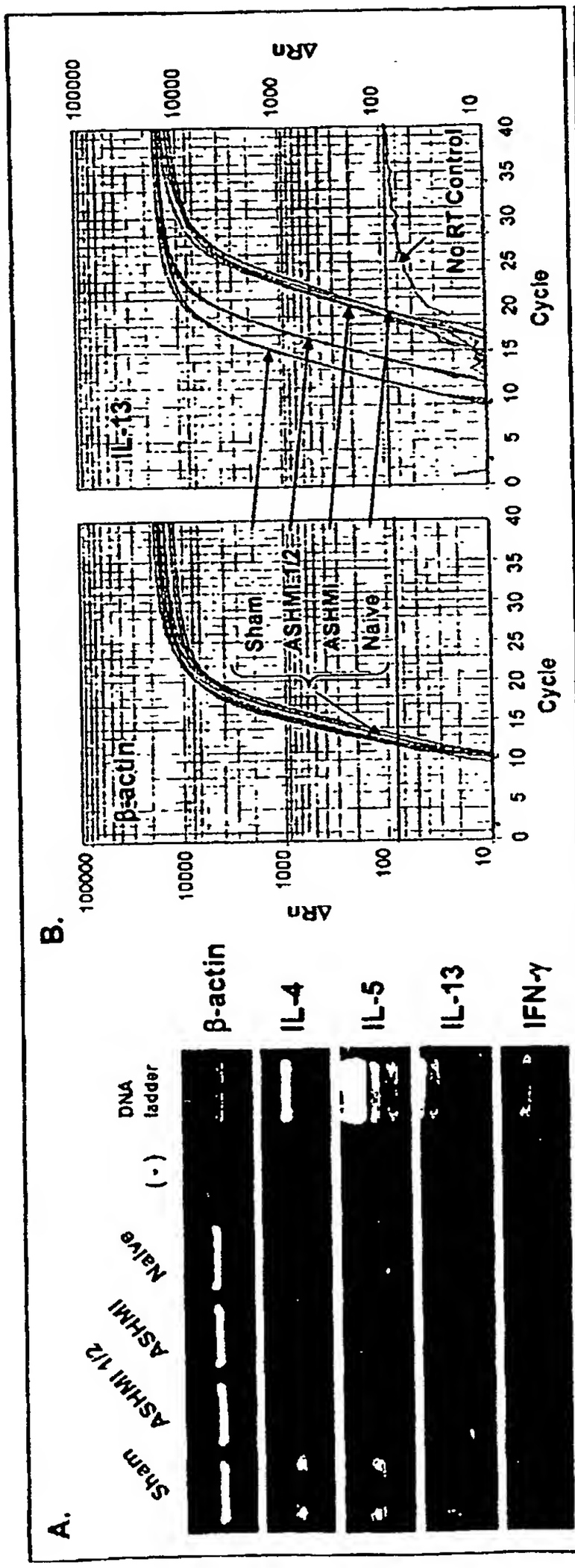


Fig 16 Semi quantitative RT-PCR. Total RNA was extracted from the lung of sham treated, ASHMI treated and naïve mice. Gel illustrates cytokine mRNA expression compared with β-actin. The results are representative of 3 separate RT-PCR experiments from 3 lungs of each group. B. Amplification plots of cDNA samples for IL-13 and β-actin. Three cDNA samples from 3 lungs of each group were performed.

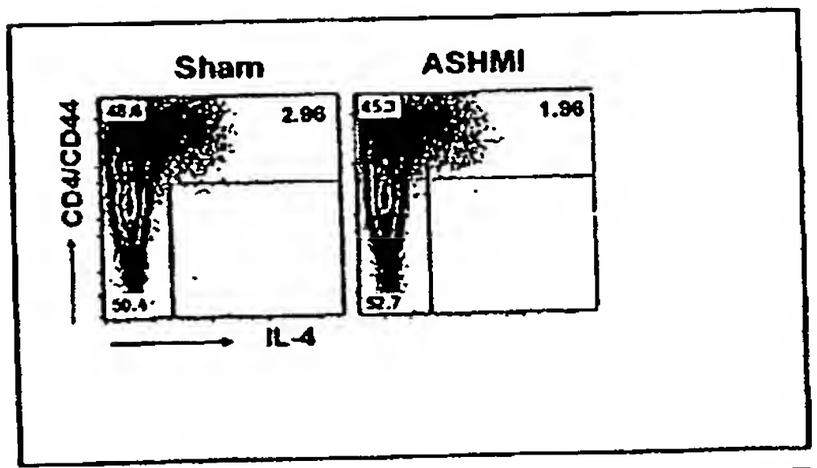


Fig 17. Flow cytometry: Data show the percent of CD4+CD44+ IL-4+ cells.

FIGURE 17

FIGURE 18

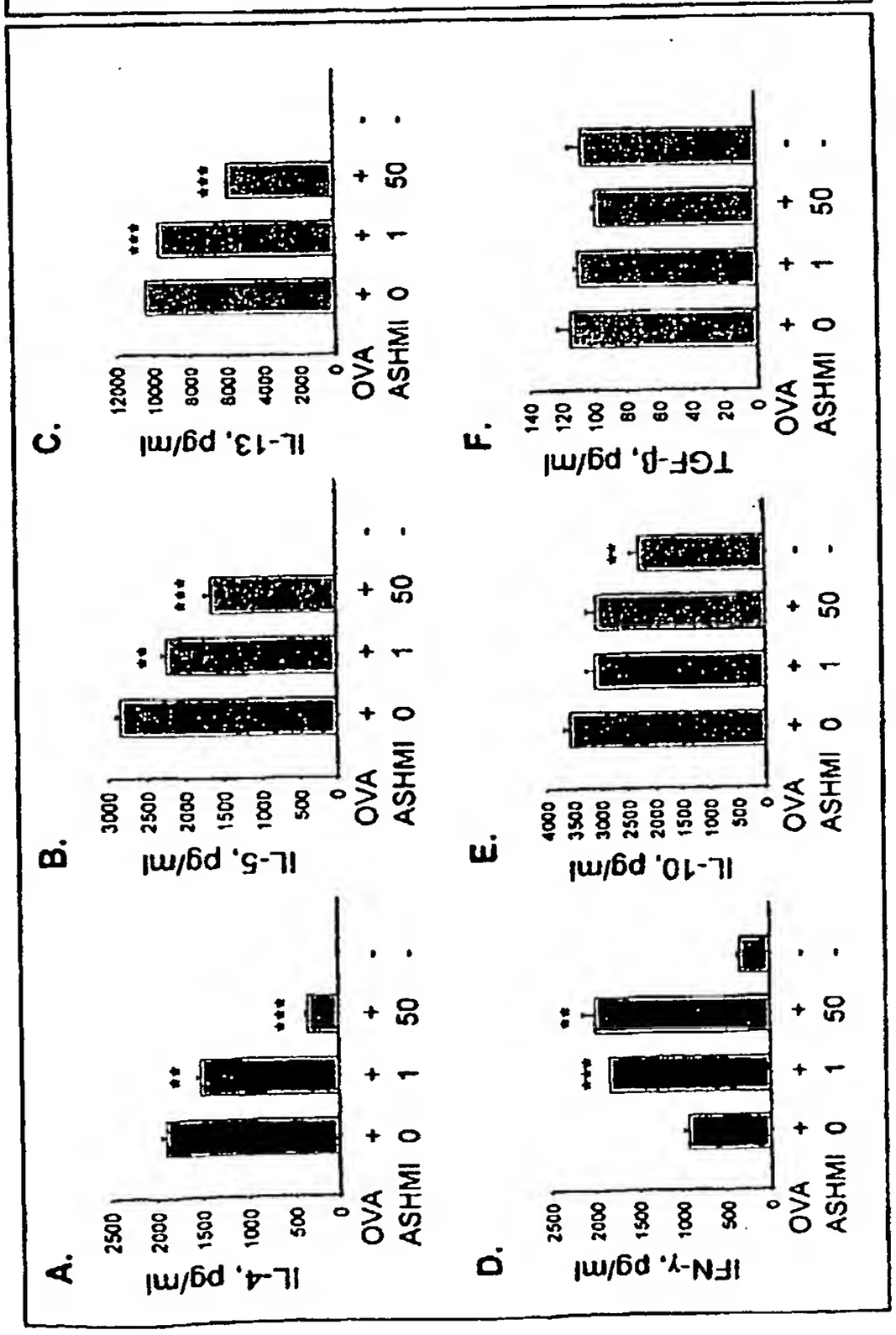


Fig 18. Effect of ASHMI on Th2-polarized splenocytes. Th2-polarized splenocytes were cultured with medium alone, or OVA in the presence or absence of ASHMI at 1 or 50µg/ml. Supematants were collected 72-hrs later, and cytokine levels were determined by ELISA. Results are expressed as mean ± SEM of 2 duplicate cultures of 4 mice. *p<0.05; ** p< 0.01,***p<0.001 vs sham.

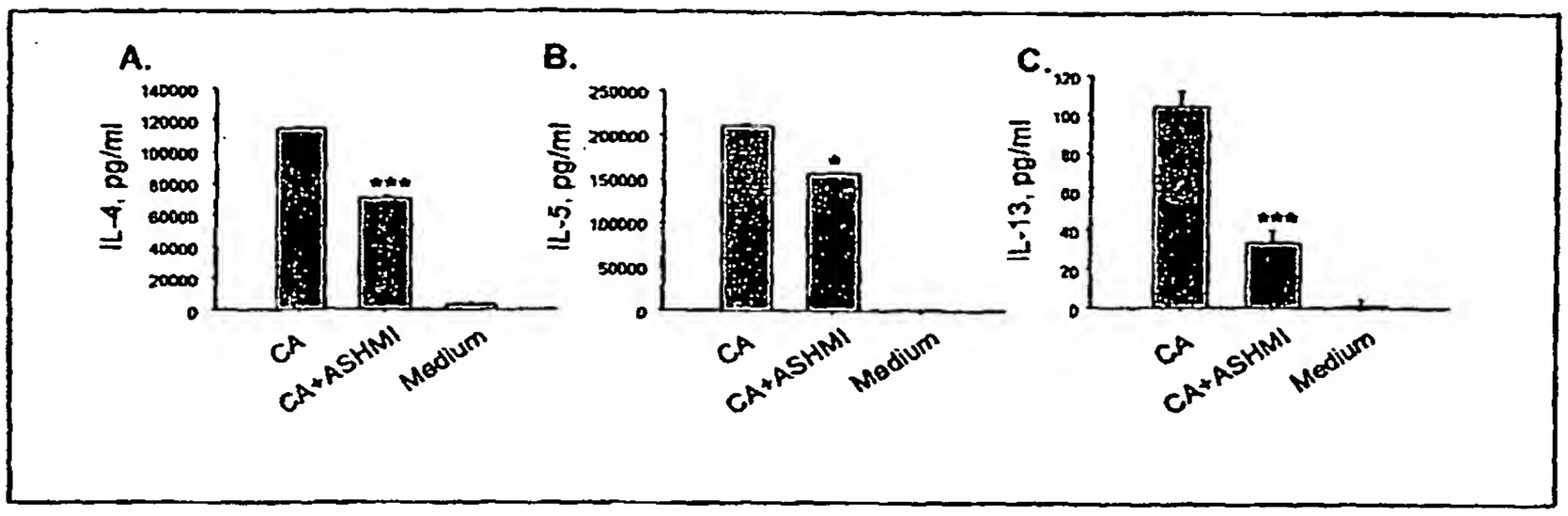


Fig 19. Effect of ASHMI on D10 cells. D10 cells were cultured in the presence of CA and irradiated syngeneic splenocytes in the presence or absence of ASHMI at 50μg/ml. Supernatants were collected 72-hrs later, and cytokine levels were determined by ELISA. Results are expressed as mean ± SEM of triplicate cultures from three experiments. *p<0.05; ***p<0.001 vs CA.

FIGURE 19

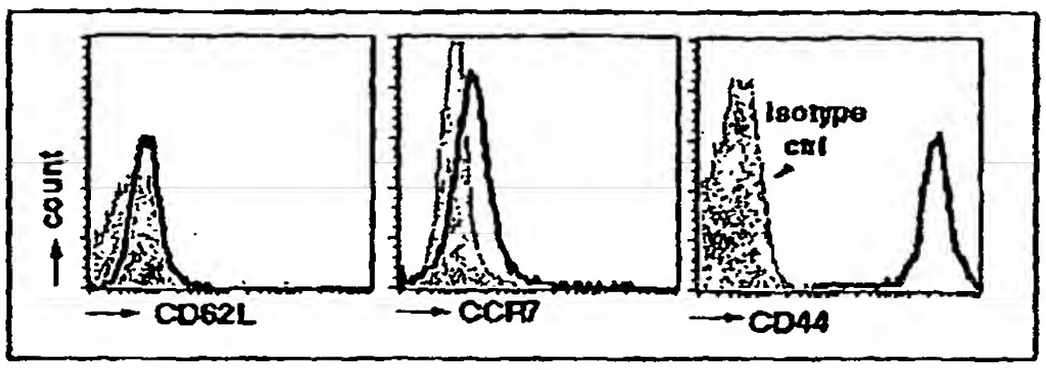


Fig 20. Phenotype of Th2 polarized cell line, D10.G4.1. Flow cytometry results show D10 cells phenotype $CD44^{high}CD62L^{neg}CCR7^{neg}$(the open histogram).

FIGURE 20

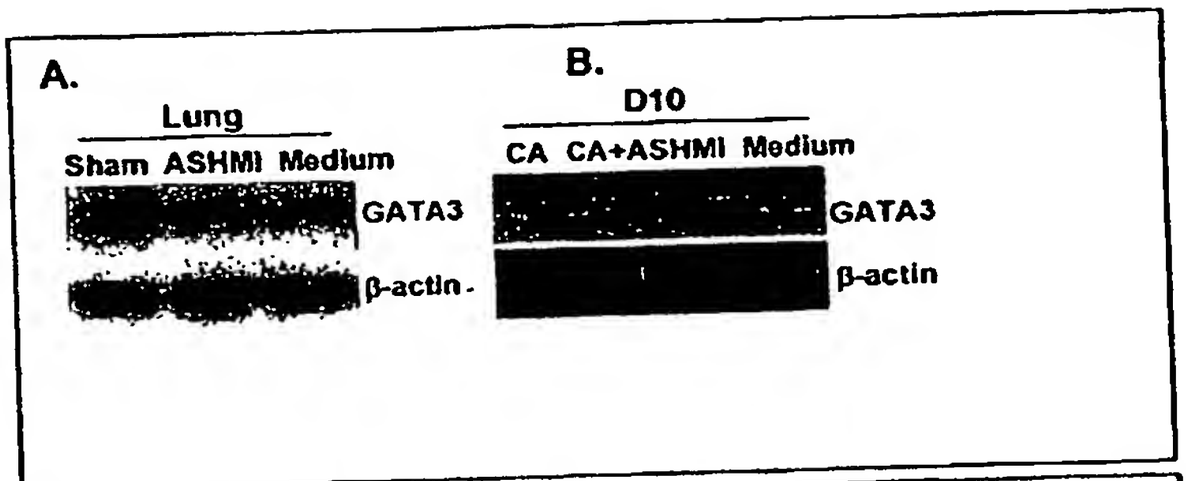


Fig 21. Western blot for determining GATA-3 protein expression in lung tissue (A) and D10 cells (B).

FIGURE 21

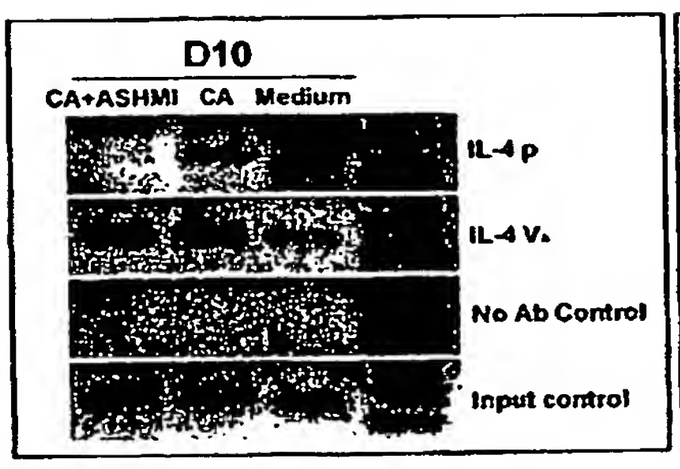


Fig 22. ChIP Assay. Illustration of ChIP assay using a ChIP Assay Kit (Upstate Biotechnology. Lake Placid NY) indicates GATA-3 binding to IL-4 promoter and enhancer in the D10 cells with or without CA-stimulation in the presence or absence of ASHMI treated cells.

FIGURE 22

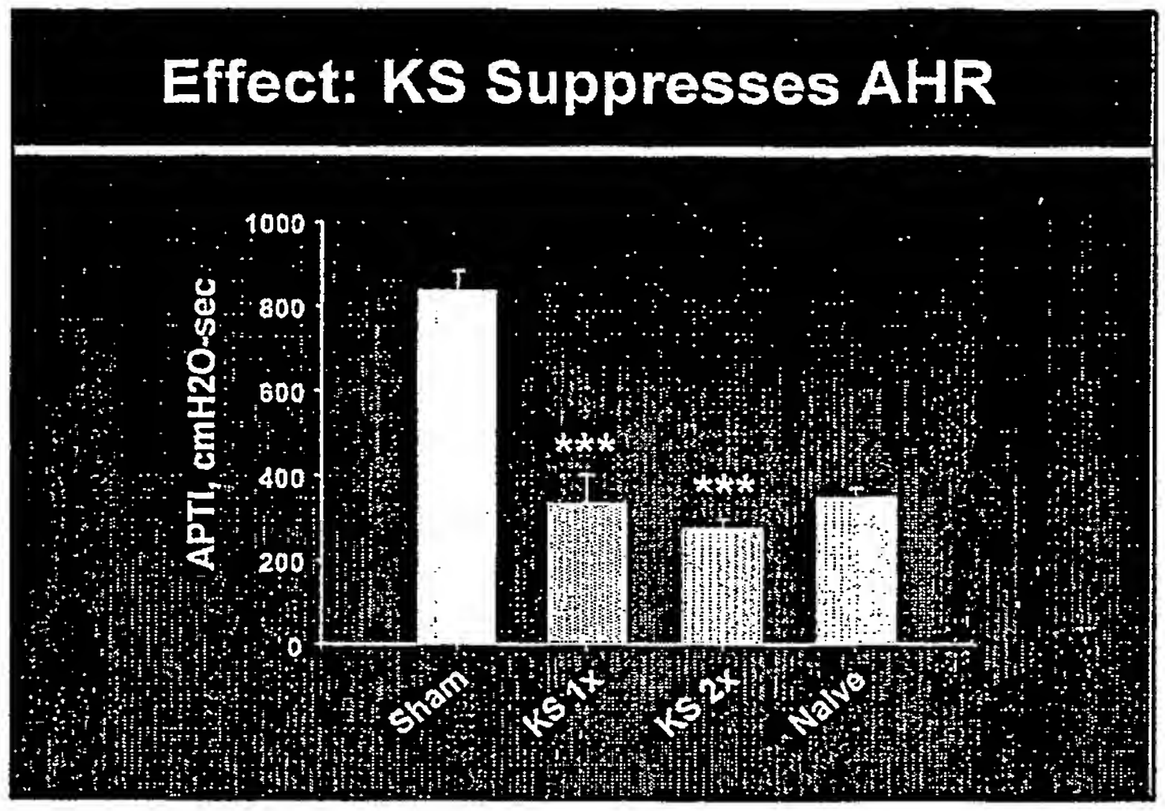

Effect: KS Suppresses AHR
APTI, cmH2O-sec
1000
800
600
400
200
0
***
***
Sham
KS 1x
KS 2x
Naive

FIGURE 23

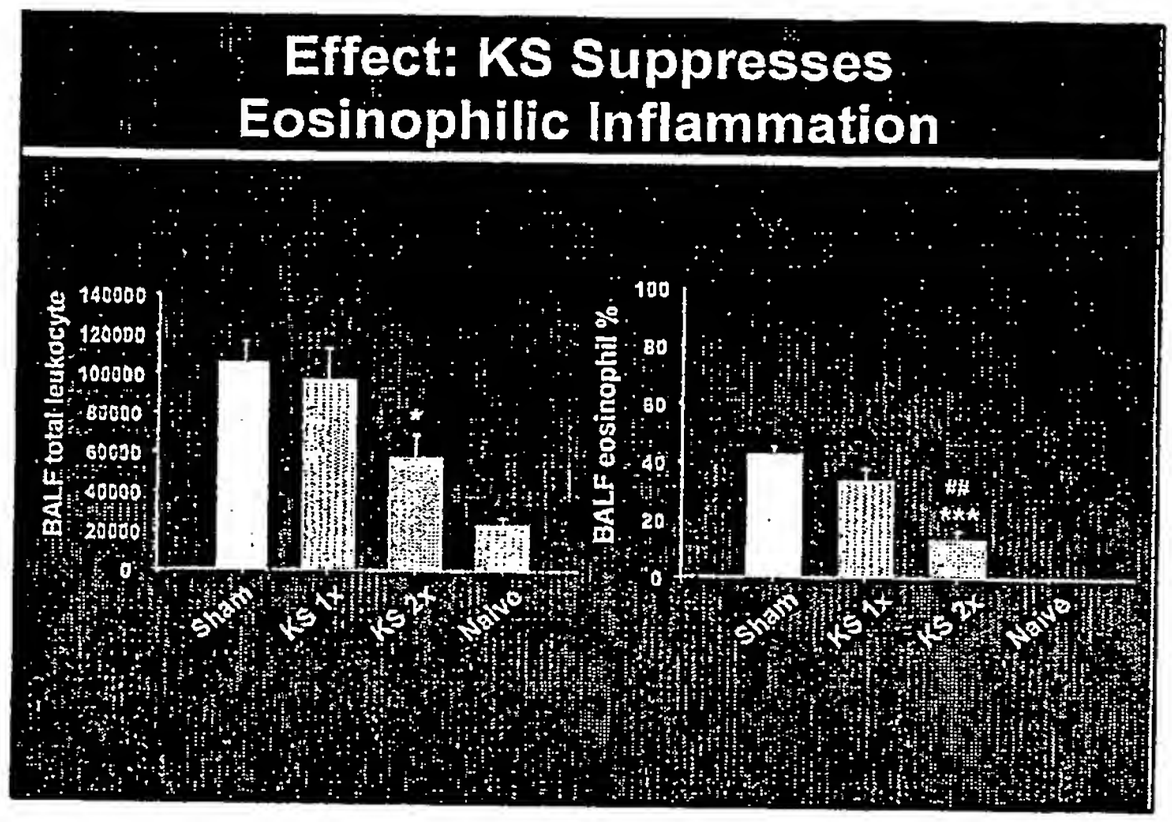

Effect: KS Suppresses Eosinophilic Inflammation
BALF total leukocyte
140000
120000
100000
80000
60000
40000
20000
0
*
Sham
KS 1x
KS 2x
Naïve
BALF eosinophil %
100
80
60
40
20
0
##
***
Sham
KS 1x
KS 2x
Naïve

FIGURE 24

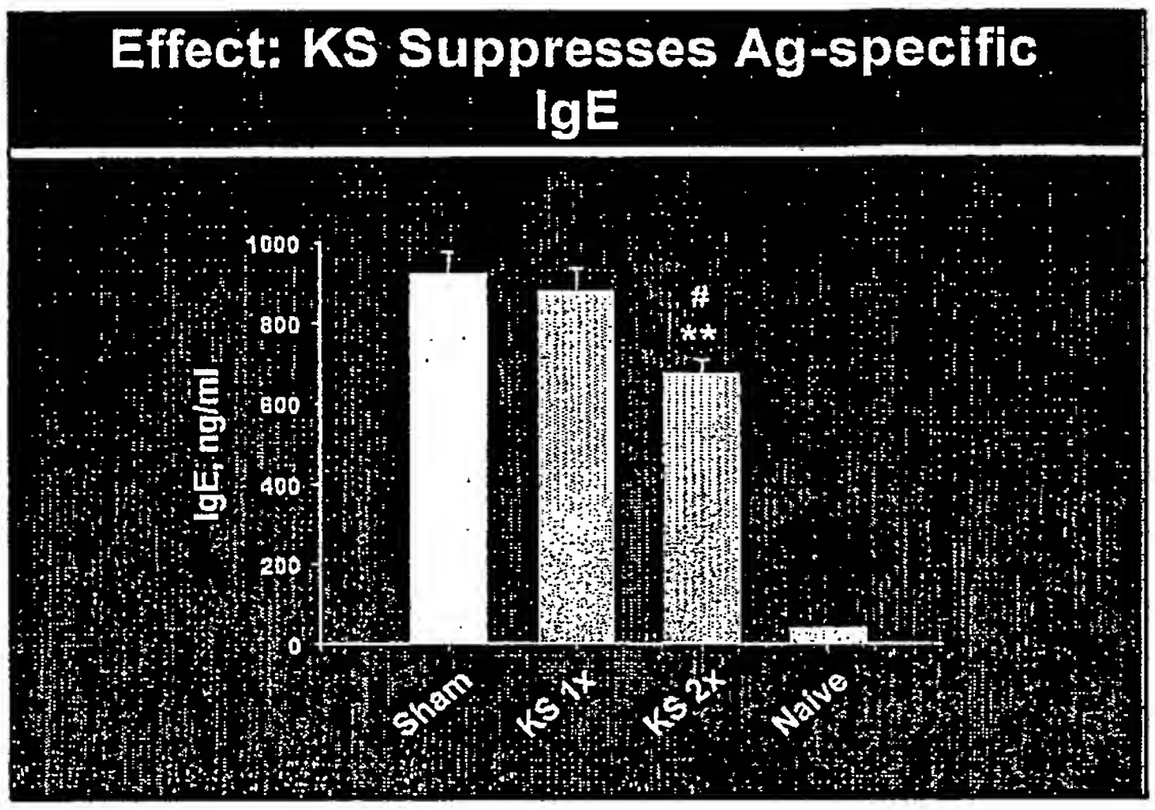

Effect: KS Suppresses Ag-specific IgE
IgE, ng/ml
1000
800
600
400
200
0
#
**
Sham
KS 1x
KS 2x
Naive

FIGURE 25

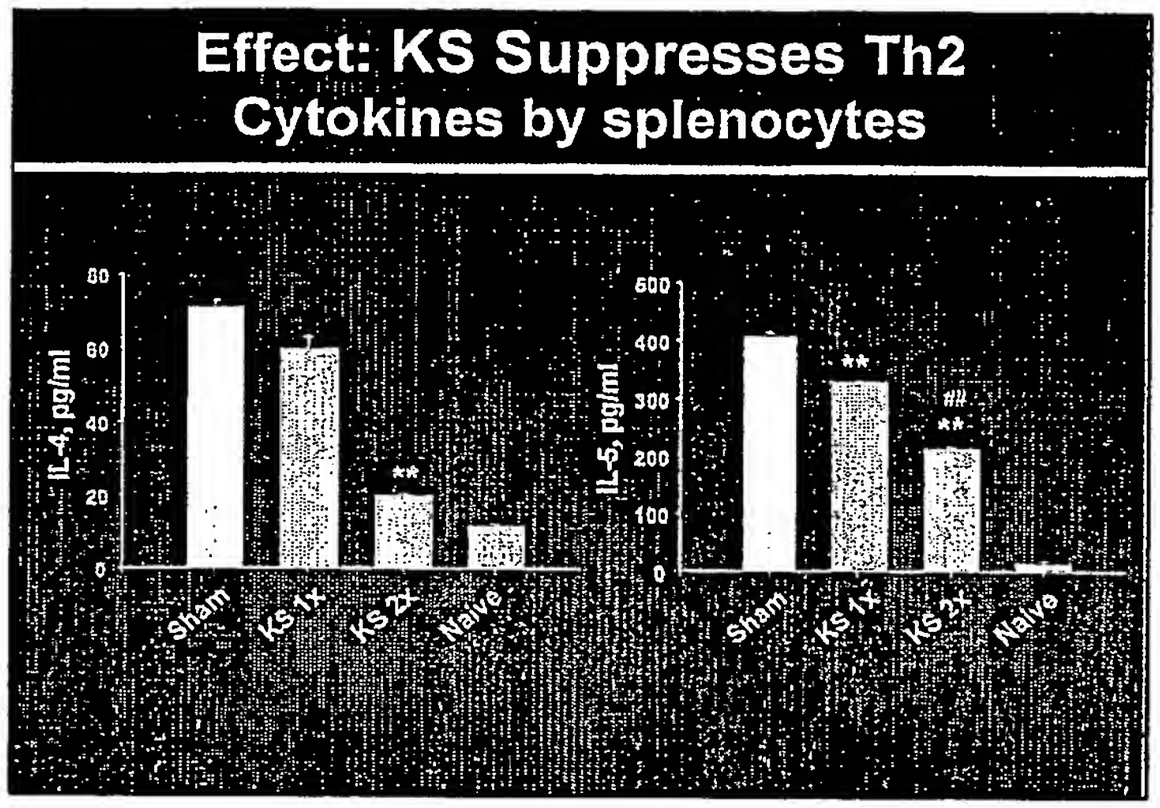

Effect: KS Suppresses Th2 Cytokines by splenocytes
IL-4, pg/ml
80
60
40
20
0
**
IL-5, pg/ml
600
400
300
200
100
0
**
##
**
Sham
KS 1x
KS 2x
Naive
Sham
KS 1x
KS 2x
Naive

FIGURE 26

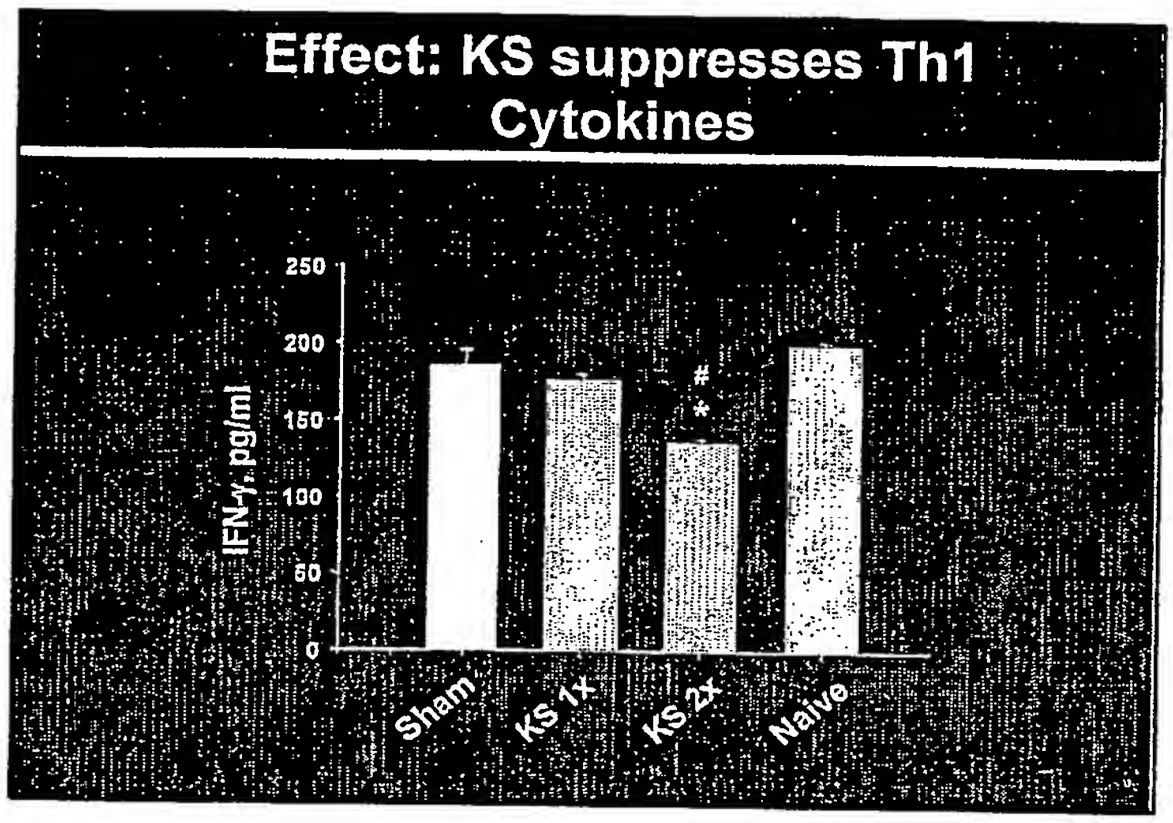

Effect: KS suppresses Th1 Cytokines
IFN-γ, pg/ml
250
200
150
100
50
0
#
*
Sham
KS 1x
KS 2x
Naive

FIGURE 27

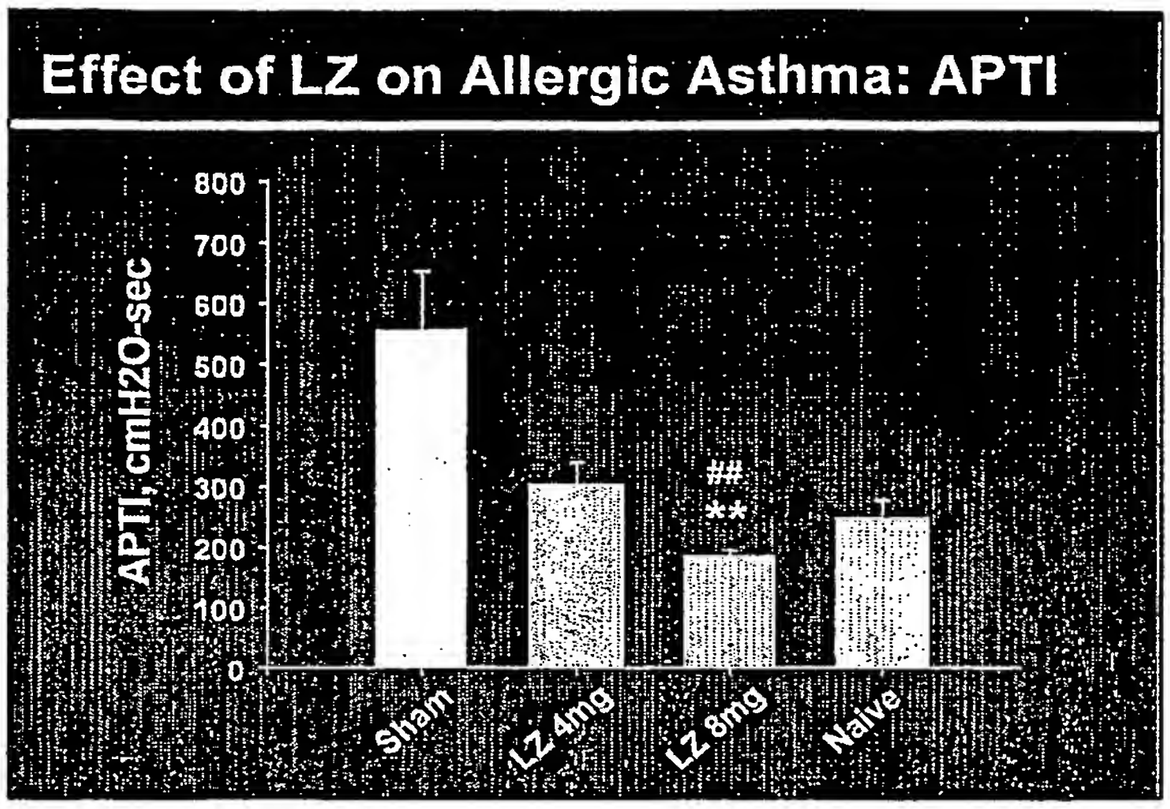

Effect of LZ on Allergic Asthma: APTI
APTI, cmH2O-sec
800
700
600
500
400
300
200
100
0
##
**
Sham
LZ 4mg
LZ 8mg
Naive

FIGURE 28

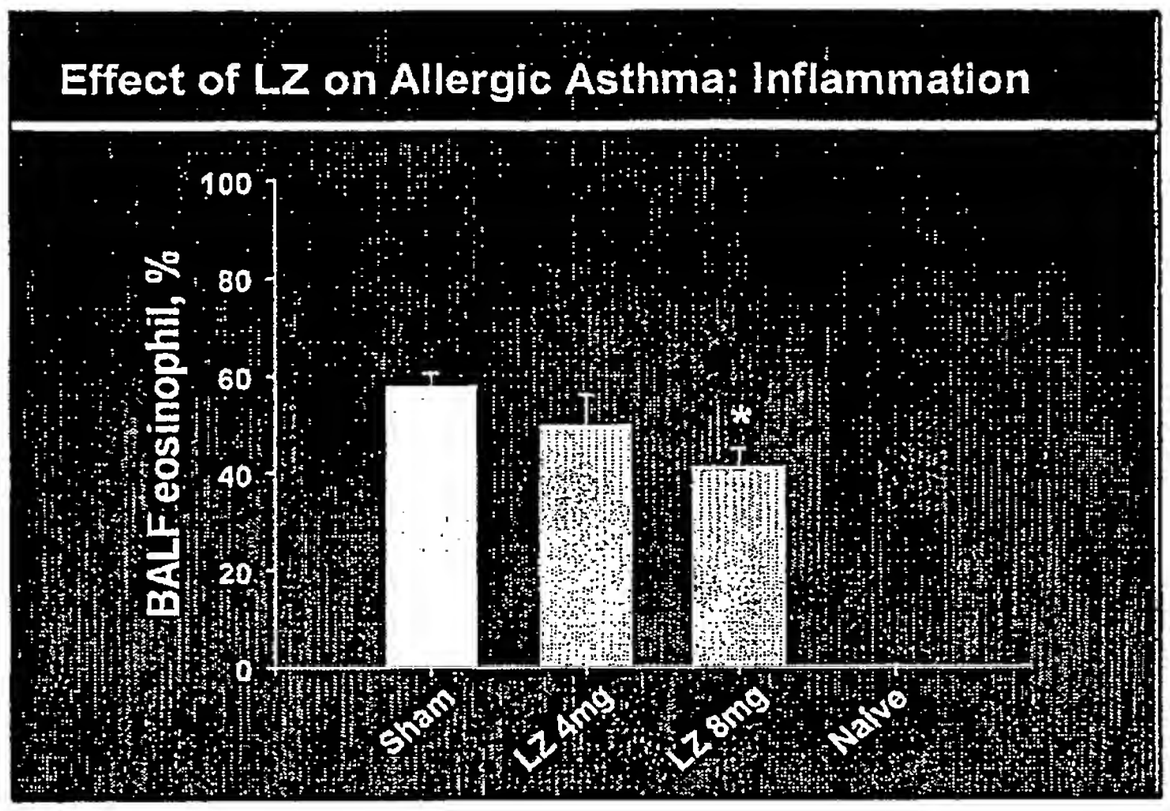

Effect of LZ on Allergic Asthma: Inflammation
BALF eosinophil, %
100
80
60
40
20
0
*
Sham
LZ 4mg
LZ 8mg
Naïve

FIGURE 29

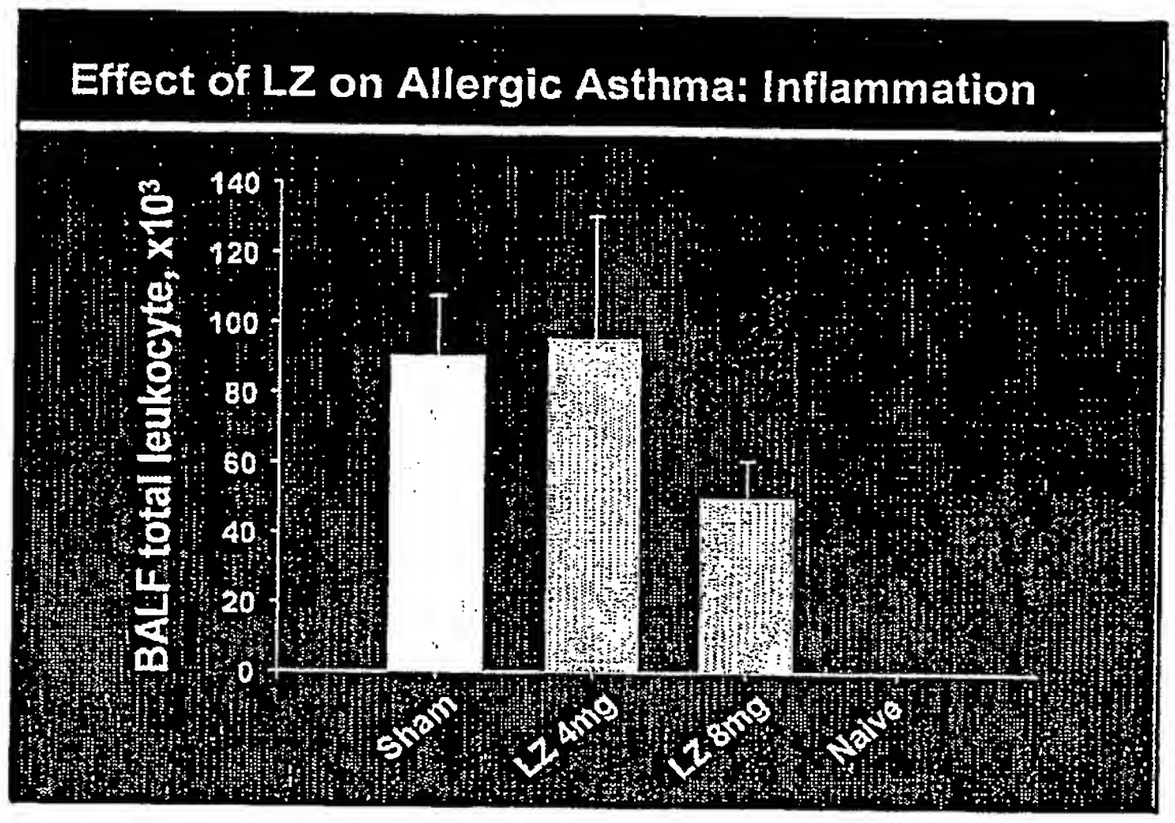

Effect of LZ on Allergic Asthma: Inflammation
BALF total leukocyte, x10³
140
120
100
80
60
40
20
0
Sham
LZ 4mg
LZ 8mg
Naïve

FIGURE 30

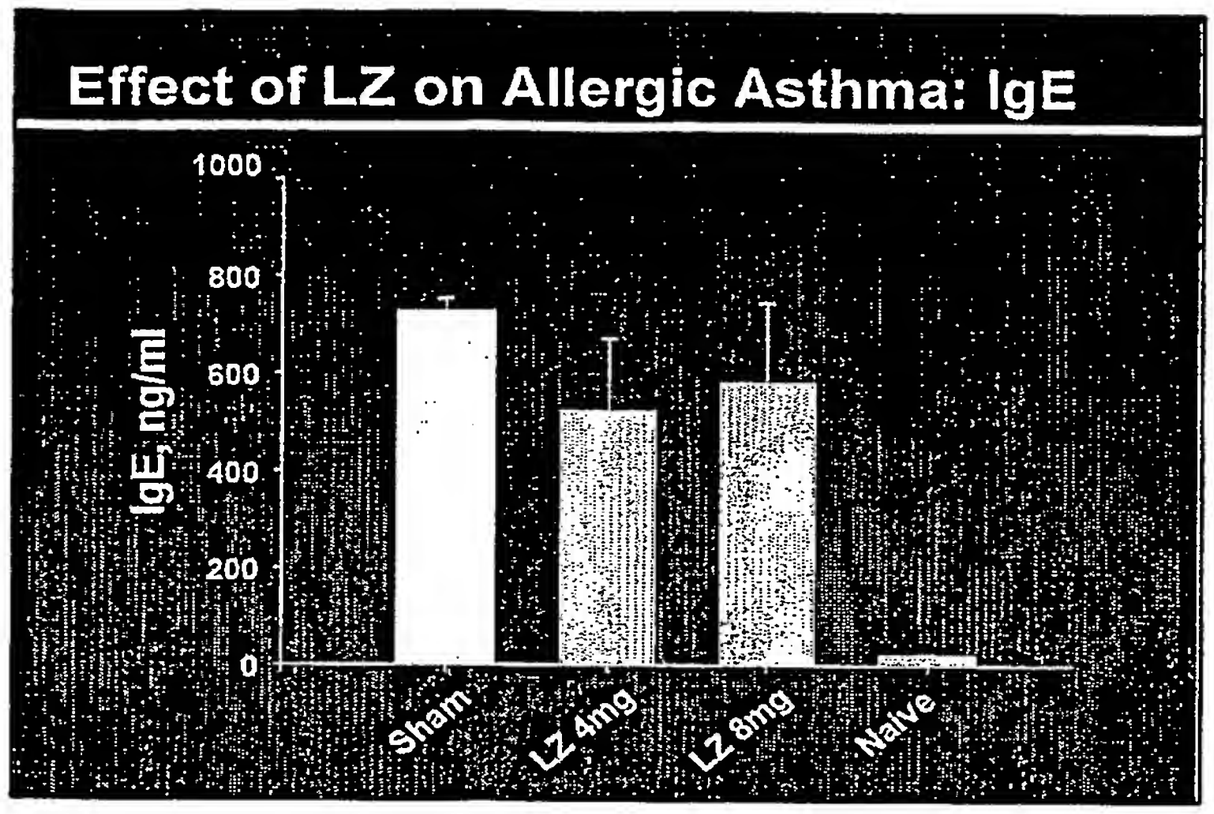

Effect of LZ on Allergic Asthma: IgE
IgE, ng/ml
1000
800
600
400
200
0
Sham
LZ 4mg
LZ 8mg
Naïve

FIGURE 31

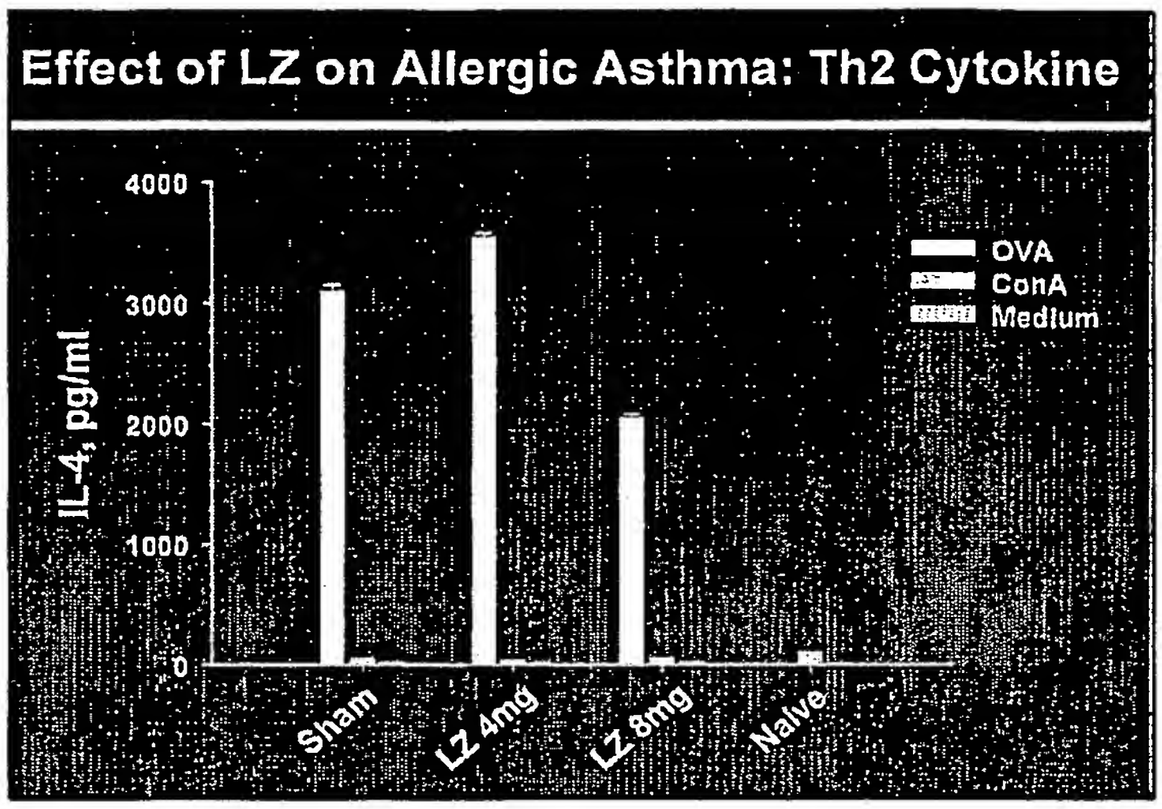

Effect of LZ on Allergic Asthma: Th2 Cytokine
IL-4, pg/ml
4000
3000
2000
1000
0
Sham
LZ 4mg
LZ 8mg
Naïve
OVA
ConA
Medium

FIGURE 32

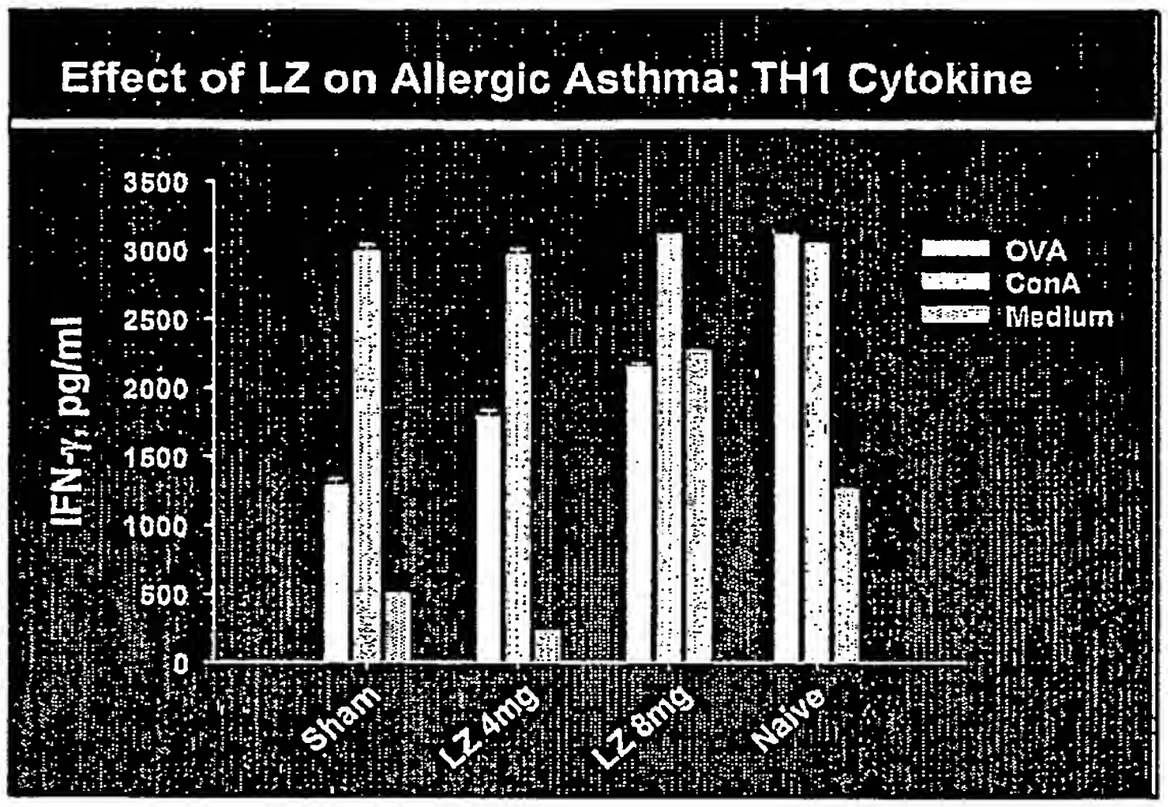

Effect of LZ on Allergic Asthma: TH1 Cytokine
IFN-γ, pg/ml
3500
3000
2500
2000
1500
1000
500
0
OVA
ConA
Medium
Sham
LZ 4mg
LZ 8mg
Naive

FIGURE 33

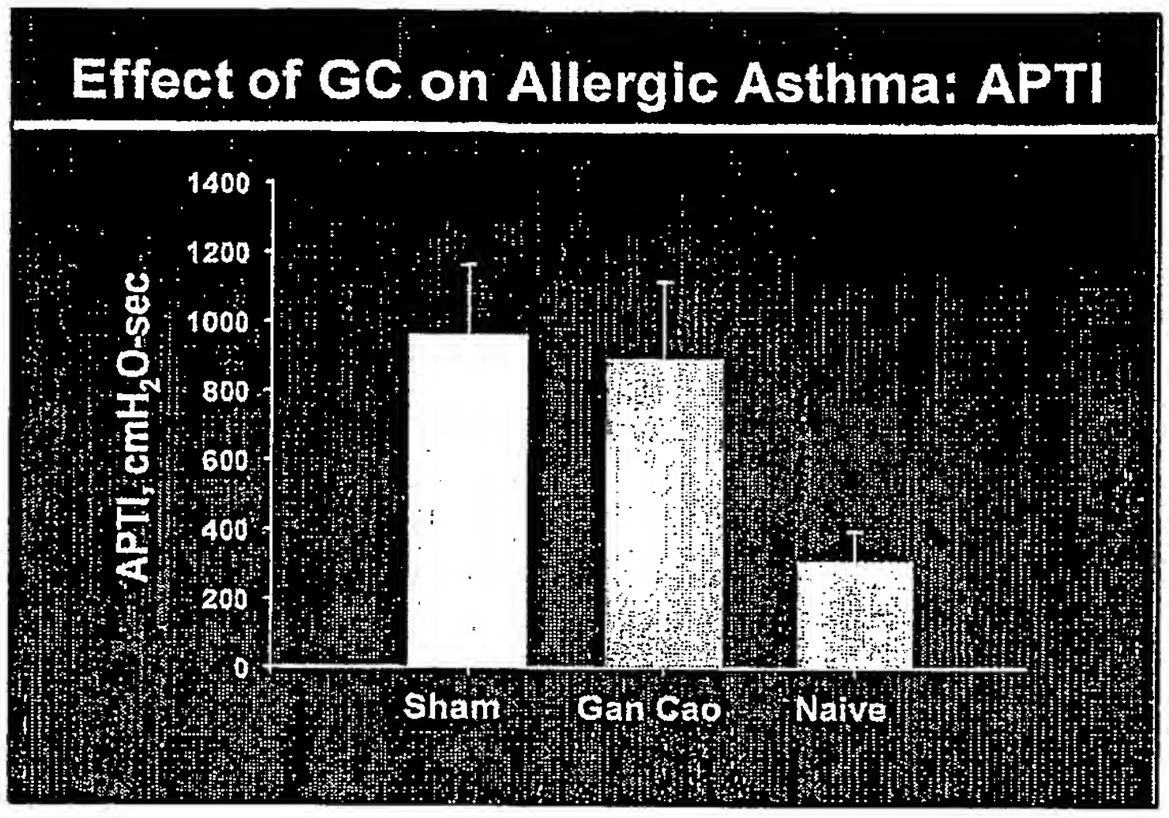

Effect of GC on Allergic Asthma: APTI
APTI, cmH2O-sec
1400
1200
1000
800
600
400
200
0
Sham
Gan Cao
Naive

FIGURE 34

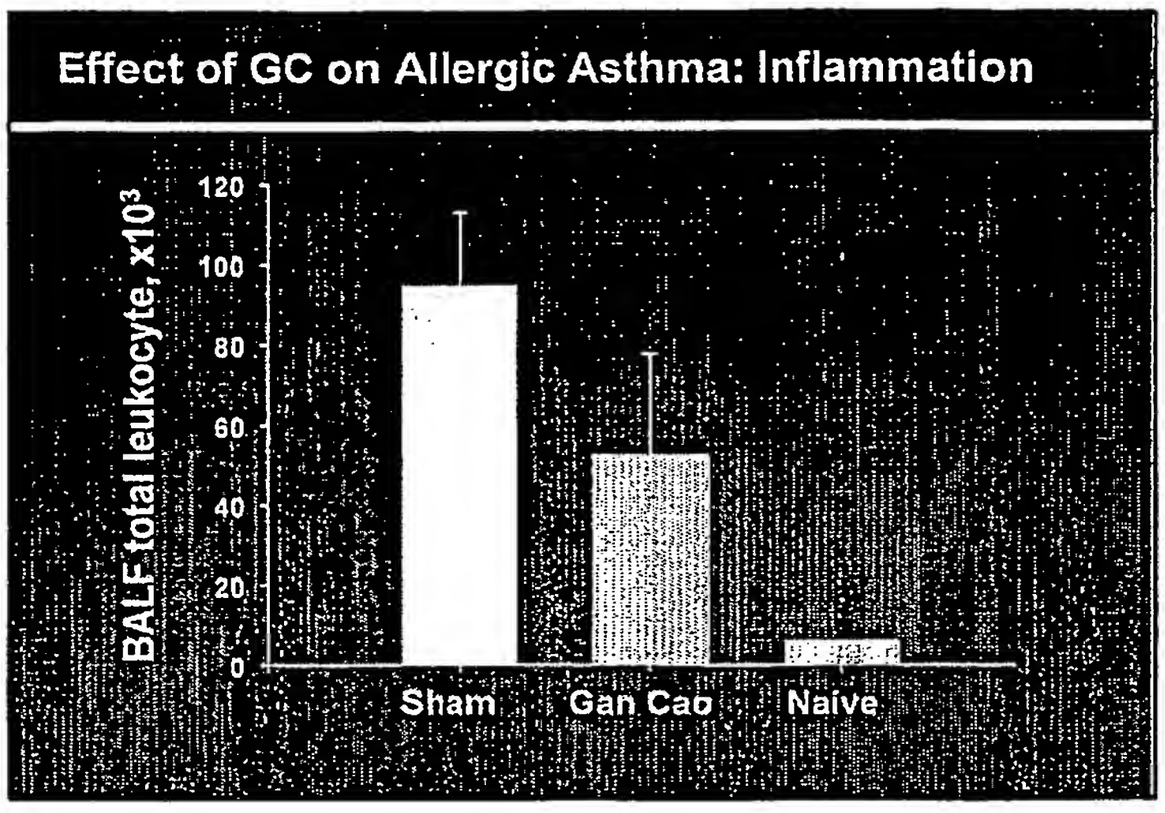

Effect of GC on Allergic Asthma: Inflammation
BALF total leukocyte, x10³
120
100
80
60
40
20
0
Sham
Gan Cao
Naive

FIGURE 35

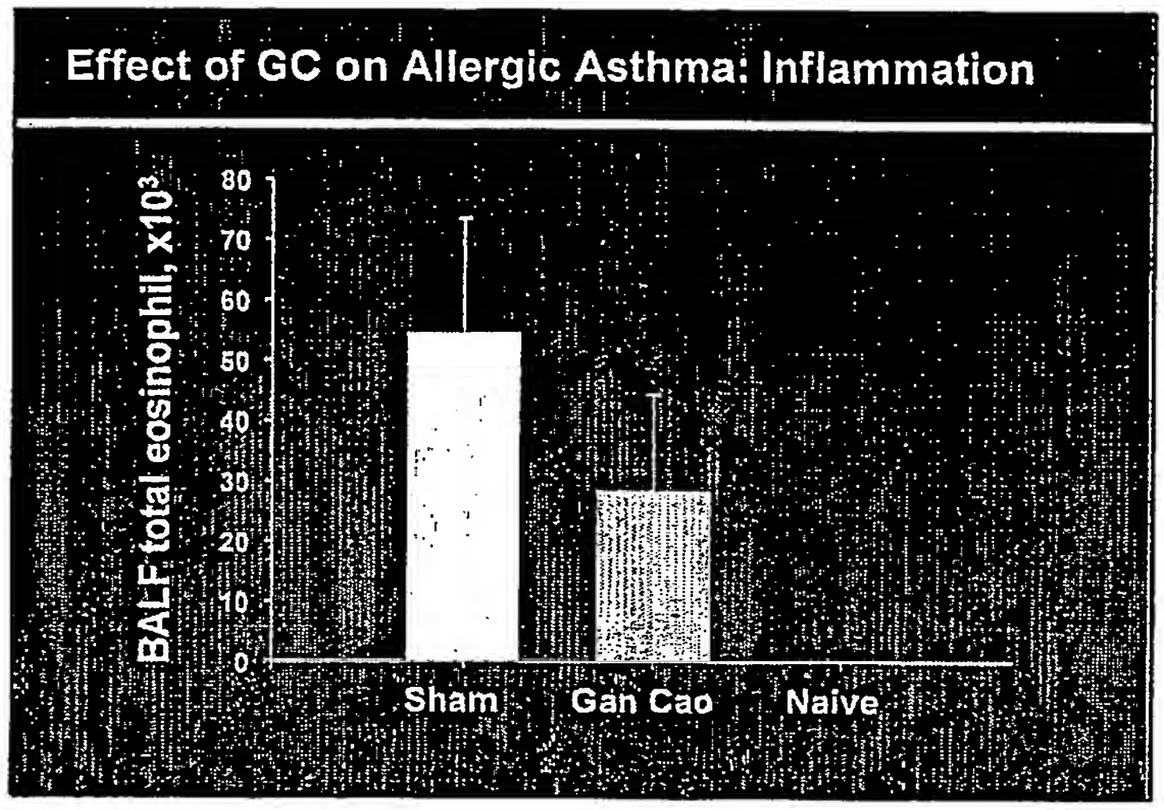

Effect of GC on Allergic Asthma: Inflammation
BALF total eosinophil, x10³
80
70
60
50
40
30
20
10
0
Sham
Gan Cao
Naive

FIGURE 36

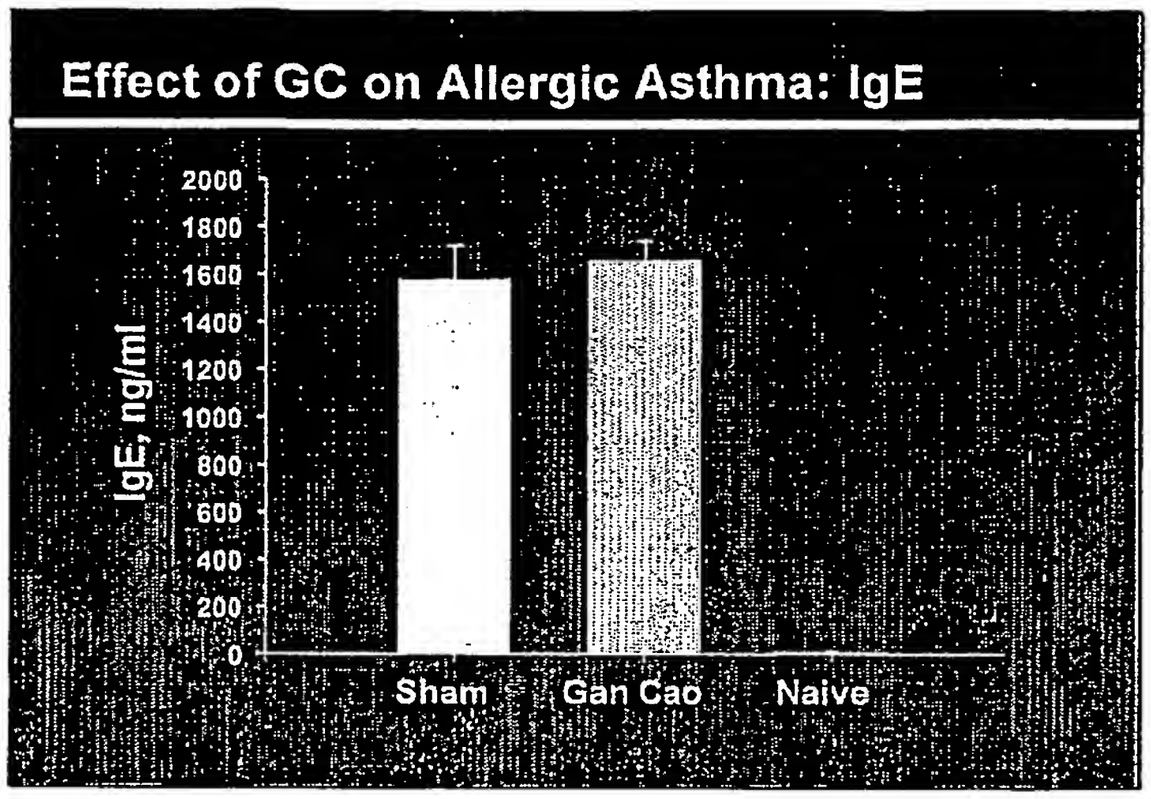

Effect of GC on Allergic Asthma: IgE
IgE, ng/ml
2000
1800
1600
1400
1200
1000
800
600
400
200
0
Sham
Gan Cao
Naive

FIGURE 37

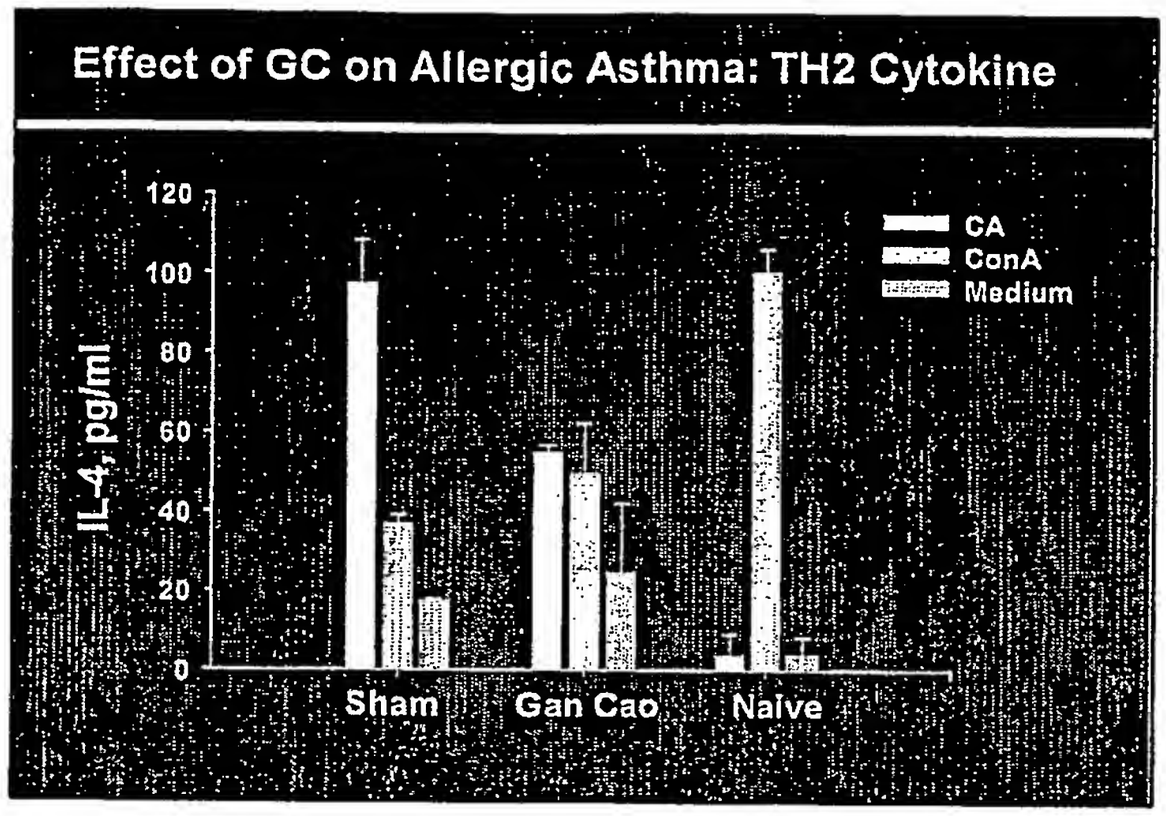

Effect of GC on Allergic Asthma: TH2 Cytokine
IL-4, pg/ml
120
100
80
60
40
20
0
CA
ConA
Medium
Sham
Gan Cao
Naive

FIGURE 38

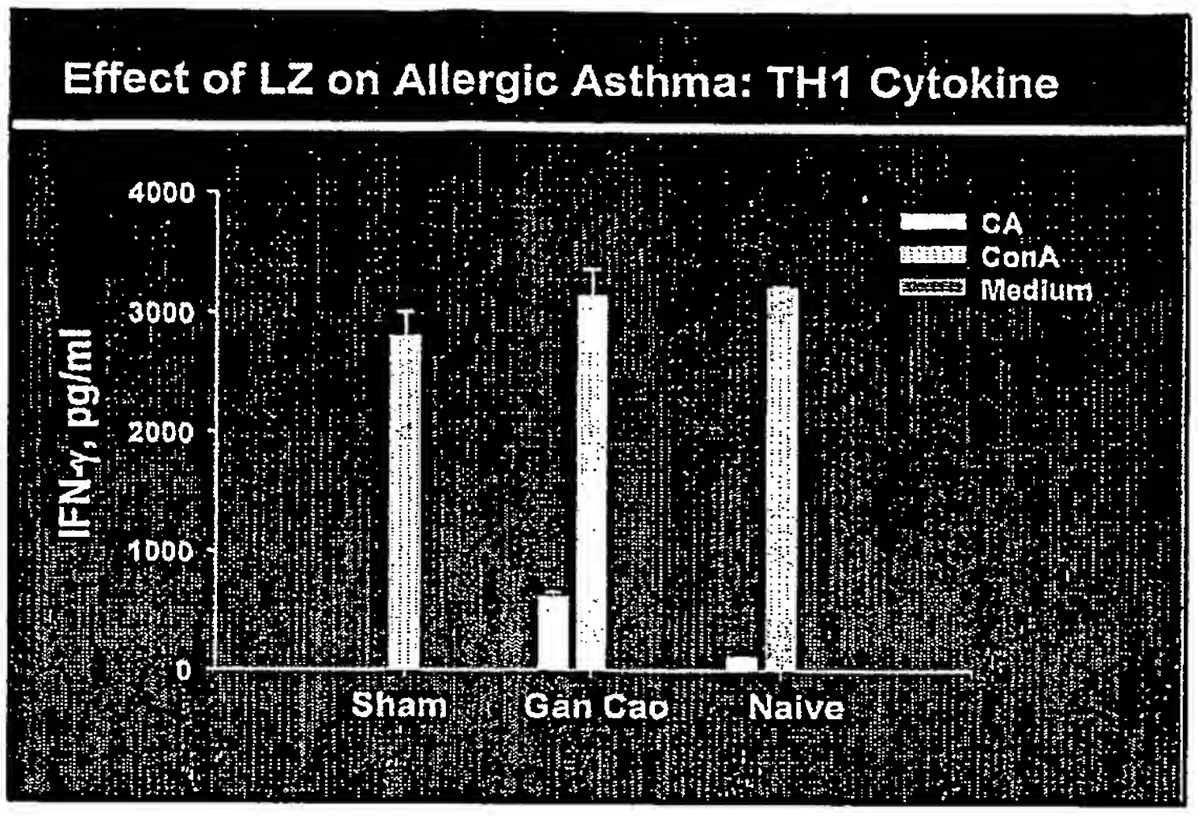

Effect of LZ on Allergic Asthma: TH1 Cytokine
IFN-γ, pg/ml
4000
3000
2000
1000
0
CA
ConA
Medium
Sham
Gan Cao
Naive

FIGURE 39

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